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**The effect of deferment length on perennial ryegrass (*Lolium perenne*) in established grazing pasture and under controlled conditions**

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

***Master of Science (Research) in Ecology and Biodiversity***

at

**The University of Waikato**

by

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THE UNIVERSITY OF  
**WAIKATO**  
*Te Whare Wānanga o Waikato*

2020

## Abstract

Perennial ryegrass is the dominant sown grass species in New Zealand, however its persistence in pasture is an issue for hill country farms. Deferring the grazing of a pasture over the reproductive cycle of ryegrass may influence changes in the above and below ground biomass of pasture plants and offers a low input and sustainable method for farmers to manage dry matter yields and quality of feed supply.

The aim of this study was to investigate the above and below ground changes in biomass and tiller production of perennial ryegrass under different lengths of deferment, after which standard rotational grazing or simulated grazing was resumed. Two trials were set up to investigate this. A field study was undertaken in an established ryegrass/clover-based pasture with three grazing treatments. Standard rotational grazing already in use on the field site (Def x0), a period of deferment over late spring/summer from November to February (Def x14) and a longer period of deferment from late spring to autumn (Def x27). After deferment, standard grazing was resumed. Above and belowground biomass, tiller densities, nutritive value, pasture composition and ground cover were measured. A glasshouse-based study investigated the effects on perennial ryegrass of different lengths of delayed defoliation and cutting treatments applied to simulate grazing. The control was cut when 2.5 to 3 new leaves per tiller had grown since the last cutting (Def x0); cutting of other treatments was delayed for an extra 4 (Def x4), 8 (Def x8) or 12 weeks (Def x12). After the delayed cutting, plants in each treatment were cut whenever there were 2.5 to 3 new leaves present per tiller. Both trials were affected by an extra-long period of rest between grazing or cutting treatments from the end of February to late April/early May due to Covid-19. This acted as an additional period of deferment for all glasshouse treatments and for Def x0 and Def x14 in the field.

The main results of the field study were that deferring resulted in higher tiller densities in winter and more accumulated dry matter before the deferred was opened for grazing than the rotational grazed control. There was also less bare ground compared with the control during summer. Nutritive value was lower during deferred periods but returned to the same value as the grazed control after standard rotational grazing resumed. There were no differences between any of the deferred treatments in root biomass. The percentage of ryegrass in total dry matter or ryegrass ground cover did not differ significantly between treatments.

In the glasshouse study, delayed defoliation increased leaf biomass, live tiller number and reproductive tiller number. Treatments with longer periods of delayed defoliation reach higher peaks in above ground dry matter and reproductive tiller number. There was a temporary increase in root biomass at depth for treatments with delayed defoliation in January. After standard defoliation resumed, Def x12 had a higher percentage of dead tillers and lower biomass by the end of the trial in July. There were no differences between treatments for live tiller number or root biomass once standard defoliation resumed.

The results suggest that a period of deferred grazing on pasture may improve dry matter yield and tiller densities of ryegrass after normal grazing resumes. The longer deferred treatment accumulated more dry matter in autumn, however it is not certain how the longer grazing rest between February and May affected potential differences between the shorter and longer deferred period. Results from the glasshouse saw no lasting benefit to ryegrass once standard defoliation resumed. Deferment length had no significant effect on belowground biomass. It is concluded that while deferred grazing may offer some benefits to a pasture, the persistence of ryegrass will not be improved by deferring grazing if other variables such as heat, moisture stress or timing of grazing are limiting potential growth. However, due to the unforeseen consequences of the Covid-19 lockdown, further research is recommended.

## **Acknowledgements**

Firstly, I wish to thank Katherine Tozer for your constant support right from the initial stages to the end. Thank you for all your hard work in making sure I had all the assistance I needed and ability to manage the work load even through the unexpected challenges of Covid-19.

Thanks also to Mike Clearwater for your part as my academic supervisor, for giving your time and guidance even when I got back to you later than ideal

I further wish to acknowledge much of the AgResearch team at Ngahere – Ruakura Research Centre for their advice and technical support of the course of this trial including but not limited to Jim Crush, Tony Craven, Martin Kear and Trevor Watson.

Thanks also must go to Lily Ouyang and Tracy Dale for maintaining space for my trial in the glasshouse and making sure the plants didn't die when I couldn't be there.

Further appreciation goes to Tim Hale for being so accommodating with the use of hill slope in the containment facility and to Aaron Malthus for being so timely with getting the cows in and out of the plots.

Thanks to Alec McGowan and Bill Carlson for their assistance with the pneumatic corer, and not giving up on getting the cores out when things got sticky.

I would also like to thank Catherine Cameron for her incredible help with the statistical analysis and kind explanations

Special thanks go to Caitlyn Dawbin and Waipaina Awarau-Morris for their part in helping shoulder the work load and making sure each harvest could be completed.

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# **1 General Introduction**

In New Zealand, the agricultural industry is largely based off the effective utilisation of pasture systems. In hill country farms this has allowed for grazing pasture and stock numbers to be reduced and for production to increase over the last decade (Beef+Lamb, 2019; Beef + Lamb; 2020). Pasture performance is closely related to animal production, and methods of improving pasture are valuable for improving economic output on farms (McLean, 2011).

Of all pasture species currently in use in the pastoral system, perennial ryegrass is dominant for its productive potential (Lee et al. 2012). However, ryegrass grows best in moist, fertile soil and can struggle to persist in the more drought prone areas of the upper North Island. (Stewart et al, 2014). This has caused pasture persistence to be identified as a key issue amongst hill country farmers (Tozer et al. 2016).

Grazing management strategies such as withholding grazing over the reproductive period of perennial ryegrass may be beneficial for tiller densities by encouraging the initiation of daughter tillers (Matthew et al, 1991) and allowing for the maturation of seed head and subsequent seedling establishment (L'Huillier & Aislabie, 1988). A link between root biomass and defoliation frequency in ryegrass has been established in previous literature (Ennik & Hofman, 1983). There is potential for roots to increase in biomass and depth in association with a period of deferred grazing. This could improve the pasture persistence of ryegrass through greater competitiveness against other species and ability to obtain water and nutrients from the soil (Hofman & Ennik, 1982; Matthew et al. 2012). However, it is still unknown how a period of deferment should be timed to best benefit perennial ryegrass. Further-more, because of the difficulties associated with studying roots belowground, there is a lack of research on the influence of deferring a paddock from grazing on roots of perennial ryegrass and pasture species in general.

## **1.1 Study Aim**

The aim of this thesis was to investigate the influence of different lengths of deferment on the above and below ground biomass of perennial ryegrass, tiller

production and nutritive value within controlled conditions and in an established pasture.

## **1.2 Thesis structure**

### **1.2.1 Chapter One**

Chapter One provides a brief introduction of the thesis topic and study aims. It also describes the thesis structure and objectives for each chapter.

### **1.2.2 Chapter Two**

This chapter is a literature review outlining the importance of the pasture system and issues faced by hill country farmers. It introduces pasture species and interactions that influence pasture performance and nutritive value. An emphasis is placed on perennial ryegrass as the species of focus in this study, and grazing management strategies that may influence above and belowground growth.

### **1.2.3 Chapter Three**

This chapter examines the influence of three different lengths of deferment on an established ryegrass-based pasture. The hypothesis tested is that aboveground dry matter and belowground biomass increases under pasture when deferred in spring and that this increase will be greater under an extended period of deferment ending in autumn.

### **1.2.4 Chapter Four**

This chapter investigates the effect of four different lengths of deferment on perennial ryegrass in controlled glasshouse conditions. The hypothesis tested is that root biomass will increase as above ground biomass increases when defoliation of ryegrass leaf is delayed, and that this increase in root biomass will be lost after standard defoliation resumes

### **1.2.5 Chapter Five**

This chapter provides a summary of the main findings, their implications and provides suggestions for future research

## **2 Literature review**

The agricultural industry in New Zealand is an important part of the economy for food production and exports. Of the land in New Zealand dedicated for agricultural use, 81% of that area was dedicated to sheep and beef or dairy farming as of 2017 (Beef + Lamb New Zealand, 2019). New Zealand farm systems are traditionally pasture based of which ryegrass-clover mixes are the dominant choice for forage (Kalaugher et al., 2017). Perennial ryegrass grows well in a range of New Zealand conditions but is best suited for moist, fertile conditions, whereas the drier conditions associated with many hill country farms particularly in the upper North Island present challenges for persistence of perennial ryegrass (Stewart et al., 2014).

New Zealand hill country farms occupy 5 million ha of land classified as having slopes greater than 15° and an elevation lower than 1000 m above sea level, 63% of this land is in the North Island (Kerr, 2016). Predominately used for sheep and beef farming, as of 2020 the grazed area of farms has decreased and the number of stock units has reduced compared with statistics from 1990. Grazed areas of hard hill country farms have reduced by 23% while profit per hectare is estimated to have increased by 84% from 1990. Increases in productivity on grazed areas of hill country has allowed for this pattern of reduced input and increased outputs. Hill country sheep and beef farms are a valuable industry generating \$8.9 billion in wool, sheep and cattle sales for the New Zealand economy in 2018-19 (Beef + Lamb New Zealand, 2020). However continued improvement in management of grazing pasture is needed to keep hill country farms economically and environmentally sustainable in a changing climate.

### **2.1 New Zealand Hill Country Pasture**

In the grass-fed farming systems that are common place in New Zealand the combined quality and dry matter yield of pasture is of high importance for overall livestock production and profitability of the farm. Previous reports on the value of improving pastures has given an estimated economic value of \$6 billion (McLean, 2011). The link between pasture production and animal production is close enough that dry matter yield has been used to estimate potential pasture performance and stocking rates (Clark et al., 2009; Macdonald et al., 2008). While

there is evidence that high performance for certain paddocks is due to overall pasture utilisation and is not just a measure of dry matter (Glassey et al., 2010), pasture productivity underpins the potential economic performance of any pasture-based farm system.

#### 2.1.1 Pasture Species

The common formation of a New Zealand pasture can typically be broken down into a few categories. High performance grasses, low performance grasses, legumes and broadleaf weeds. Sown species performance can vary with suitability between regions but most pasture are based around a few species from a pool of about 25 exotic species of which perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), are popular choices, and are commonly sown together (Kemp & López, 2016).

Perennial ryegrass is therefore the dominant species in New Zealand pastures, and genetic improvements in this species over the years has made this grass a large contributor to the economic development of the agricultural industry (Lee et al., 2012). Together with white clover, these two species have a high potential for herbage accumulation and nutritive value when growing in moist, fertile conditions with a temperature range between 7-20°C. Ryegrass and clover compete strongly for light against other species when growth is unrestricted through a high, relative growth rate. However, both species struggle in dry conditions or temperatures above 30°C which are often associated with upper North Island hill country farms, particularly during summer months (Chapman et al., 2011; Kemp & López, 2016).

Legumes such as white clover are often sown alongside a grass species to improve pasture productivity with their nitrogen fixing ability. Legumes can improve the available nitrogen for non-nitrogen fixing grasses. Additional nitrogen is needed for pasture performance because New Zealand soils are naturally low in available nitrogen (Maxwell et al., 2016). Nitrogen is a key nutrient, and a lack of N limits plant production (Sun et al., 2008). Combined legume/grass based pasture systems helps with the efficiency of N uptake and can keep production high while lowering the need for fertiliser inputs (Ledgard, 2001)



Dry hill country areas may be sown with a mix of species that are more drought tolerant as an alternative to ryegrass, but these can come with other downsides such as difficulty sowing or tolerance of stock grazing and trampling. Common grasses that may be sown instead of ryegrass include cocksfoot (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*) which are slower to establish than ryegrass as well as phalaris (*Phalaris aquatica*) which has good drought tolerance but can produce toxic alkaloids (Tozer & Douglas, 2016).

The forage herbs like chicory (*Cichorium intybus*) or plantain (*Plantago lanceolata*) may also be sown in mixtures for dry hill country (Morris & Hickson, 2016). Herbs are often preferred in combination with other species as herb species alone can have lower productivity than mixed swards (Cranston et al., 2015). Plantain has been reported to often increase in proportion following a drought and contributes the most to a pasture when grass or legume growth is poor and there are gaps in the pasture sward (Stewart, 1996). A common legume substitute for white clover is sub-clover which has also been successfully used on dry hill country farms (Cranston et al., 2015)

### 2.1.2 Industry Challenges

Hill country farms remain under pressure, increased by climate change, to meet economic and environmental goals while aiming for a sustainable agricultural system (Dodd et al., 2020). This results in a continued need for new ways that farmers can improve their use of grazing pasture. Problems associated with poor pasture management of hill country include lack of growth as well as increased risk of erosion and nutrient runoff, both of which negatively affect production and sustainability (Nie & Zollinger, 2012). Improved growth of pastures may help farms increase animal production but management practices must balance the economic demand with sustainable practices that reduce negative environmental impacts (Baskaran et al., 2009; White et al., 2010).

### 2.1.3 Issues impacting pasture performance

Pasture persistence has been identified as a key issue amongst hill country farmers due in part to the link between high performing pasture and overall farm economic performance (Tozer et al., 2016). Some non-grazing stresses on hill

country pasture that are likely to impact performance in New Zealand are drought, weed invasion and invertebrate pests.

The impact of moisture deficits on species establishment and persistence has been an ongoing concern, especially for hill country farmers in a changing climate.

While drought over summer is common for Northland and parts of the east coast of New Zealand, recent years have seen more climatic extremes with three of the past five years being between the warmest and 4<sup>th</sup> warmest years on national record and more widespread moisture deficits over summer. The pre-trial summer of 2018/2019 was the 4<sup>th</sup> warmest year on record (NIWA, 2020). The annual 2019 rainfall was 50-79% below normal rainfall and resulted in higher than normal soil moisture deficits in January and February for much the country particularly in the upper North Island regions as well as Nelson and Tasman regions in the South.

The 2019/2020 summer did not have the record-breaking temperature of the previous year but also saw record or near record low rainfall for much of the North Island and northern South Island regions. Severe drought was recorded in Northland and Auckland regions according to NIWA's New Zealand Drought Index (NIWA, 2020).

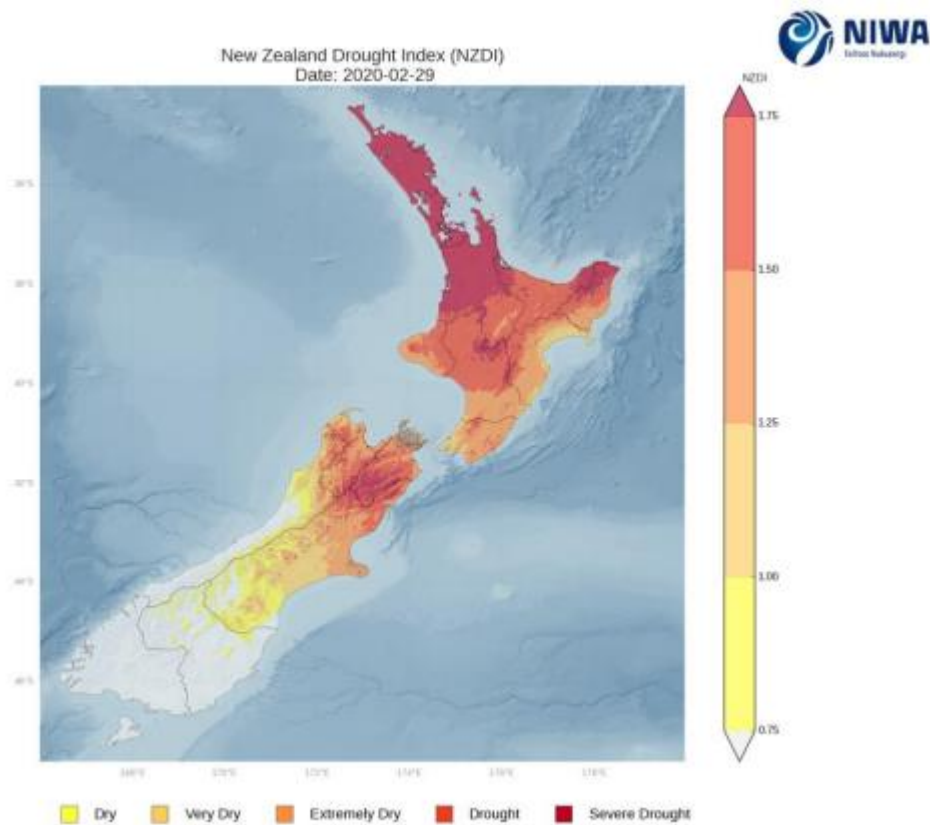


Figure 2.1: A visual map of the New Zealand regions impacted by drought in the 2019/2020 summer (NIWA, 2020):

When climatic extremes limit the growth of sown pasture species, potential is opened up for weed ingress to occur (Ramesh et al., 2017). There are up to 245 plant species from 40 different families that have been identified as pastoral weeds. Weeds are a problem to pasture systems as they compete with and replace sown species, reducing pasture quality and yield, lowering the overall performance of pasture and the efficiency of land use (Ghanizadeh & Harrington, 2019). Certain invasive weeds also have toxic alkaloids which are harmful to the health of grazing livestock. For example, ragwort (*Jacobaea vulgaris*) alone has been estimated to potentially have cost the farming industry \$64 million in 2015 if no control was implemented (Fowler et al., 2016). For many hill country farms, one of the most problematic weeds has been the Californian thistle (*Cirsium arvense*) which grows rapidly and restricts available grazing area and pasture yield (Chalak - Haghighi et al., 2008). A common control method for weeds has been the application of herbicides, particularly glyphosate, however other strategies are needed due to increasing plant resistance to herbicides. Alternative include the use of biocontrol agents like the beetle (*Cassida rubiginosa*) which

has been used on thistles and grazing management strategies that uses timing of grazing, intensity of grazing and stock class to influence pasture growth and composition (Cripps et al., 2019; Harrington et al., 2016).

An additional cause of stress on pasture are invertebrate pests which can have a damaging impact on the persistence of New Zealand pastures. Invasive species found in pasture include the clover root weevil (*Sitona lepidus*), the larvae of which feeds on clover roots and nodules. White clover is particularly vulnerable to this pest, with the effects resulting in reduce foliage. The Argentine stem weevil (*Listronotus bonariensis*) is also considered a damaging pest to ryegrass as well as cereal crops. This weevil eats the stem and leaves, reducing yield and potentially increasing seedling death (Goldson et al., 2005). New Zealand also has endemic species that negatively impact on the pasture system, in particular the grass grub (*Costelytra zealandica*) the larvae of which feed on roots of both grass and clover and the Porina moth (*Wiseana* spp. (Lepidoptera) which feeds on the foliage of grasses and clover (Zydenbos et al., 2011). The overall impact of invertebrates on pasture systems is not easily calculated due to the variation in abiotic and biotic interactions, but has been estimated as a loss of \$1.7-2.3 billion per year for the New Zealand farming industry (Ferguson et al., 2019). With the economic losses caused by decreased pasture performance, finding low cost ways to improve and maintain pastures will be highly beneficial to farmers. Adjusting grazing management to support pasture persistence offers one such possibility.

#### 2.1.4 Nutritive Value

A separate measure for considering pasture performance in relation to animal production is to consider the nutritive value. Nutritive value gives an indicator of the pasture quality and the efficiency with which livestock will digest and use the forage consumed (Lambert & Litherland, 2000). One method of measuring pasture quality at a given point in time is through the use of near infra-red spectroscopy (NIR) analysis to estimate nutritive value. Since its introduction NIR has been used widely for agricultural research and by farmers to monitor the quality of forage as well as digestibility for livestock (Corson et al., 1999). The overall quality of the feed is determined by both the intake by livestock and nutritive value. Nutritive value measures components such as available macro-

and micro-elements, crude protein (CP), fibre content and metabolizable energy (ME). Different species will vary in nutritive value and legumes in particular have higher nutritive value than grasses as they are usually lower in fibre and higher in protein. This means pasture quality is affected by both species composition of a pasture and any factors that influence growth such as soil fertility, climate or reproductive stage of plants (Waghorn & Clark, 2004).

Energy predictions provided by NIR analysis is an indicator of the ME that the feed will provide to livestock. Dietary ME provides the necessary energy needed as a part of maintenance and production for animals. It has become a popular estimate of feed quality in New Zealand as available energy in pasture feed is one of the limiting factors to animal production on New Zealand farms. Use of ME predictions alone is not a good indicator of feed quality because it doesn't give information on nutrient content or efficiency of digestion by grazing animals (Waghorn, 2007).

Another commonly used indicator of feed quality alongside ME is neutral detergent fibre (NDF). This is a measure of the fibre content available in feed based on the plant cell wall. It is an important part of feed as fibre is needed in the diet for rumen function in cows, however too much NDF will limit feed intake especially when digestibility is low. This makes NDF content as well as digestibility important to consider when determining feed value (Oba & Allen, 1999).

Crude protein (CP) is a measure of nitrogen content and combined with NDF affects digestibility and accounts for 65-75% of potential ME. NDF and CP content also account for up to 70% of overall dry matter as low NDF is usually mutual with high CP content and vice versa. Increased CP content has a positive effect on animal production. However, at levels exceeding animal requirements high CP can become detrimental to production as energy is expended on converting excess CP to urea for excretion (Reid et al., 2015; Waghorn, 2007).

Several plant factors will affect nutritive value. Seasonal affects happen as pasture species mature, which causes nutritive value to decrease, this decrease affects grass species more extensively than legume species (Waghorn & Clark, 2004).

Crude protein can be affected by grazing management, in several grass species CP increases in response to cutting and N supply (Pontes et al., 2007). Nutritive value will also change with pasture species composition. Comparison of perennial ryegrass and white clover with several broadleaf plant species including chicory and plantain along with less desired species such as buttercup and dock found higher mineral content in the broadleaf species. Though not all species, such as buttercup are palatable to livestock this does suggest some variability in pasture species is useful for animal health and production (Harrington et al., 2006). Plant characteristics can reduce voluntary intake or palatability to animals including chemical characteristics such as tannin content in buttercup or alkaloids in ryegrass and physical characteristics such as leaf/stem ratio. These characteristics may be separate from common nutritive value measurements but also affect the value of pasture for feed as the intake is a component of the forage value to the animal (Stone, 1994).

#### 2.1.5 Methods for improving pasture persistence

Several strategies have been put forward to address the different factors that affect pasture performance. This includes the use of other pasture mixes or species in environments that are less suitable for ryegrass/clover such as phalaris or lucerne, which have deep root systems that allow for good drought tolerance (Milne, 2011). Alternatively, breeding of new ryegrass cultivars for traits that will contribute to its survival in hill country conditions has been an area of development (Easton et al., 2011). Management strategies are also important to pasture persistence including meeting nutrient needs with effective fertilizer inputs (Fraser et al., 2011), predicting pest outbreaks (Bell et al., 2011), or adjusting grazing management to limit over-grazing effects and support plant regrowth (Stevens, 2011).

The effects of defoliation on ryegrass tillers or clover stolons is an important consideration when managing pasture for improved persistence. There is potential to do this by adjusting the timing of grazing periods (Edwards & Chapman, 2011). Since perennial ryegrass is a highly utilised species in New Zealand with good productive potential (Lee et al., 2012), methods that will assist the pasture

persistence of ryegrass are of additional interest. By understanding the growth habits of perennial ryegrass, grazing strategies can be adjusted to suit.

## 2.2 Perennial Ryegrass

Perennial ryegrass (*Lolium perenne*) was introduced to New Zealand in the early 19<sup>th</sup> Century and has since become a dominant pasture species of choice for pastoral farming (Lee et al., 2012). Plant breeding has allowed for improved productivity and variations in morphological characteristics of ryegrass cultivars, such as pulling tolerance (Thom et al., 2003).

### 2.2.1 Morphological characteristics.

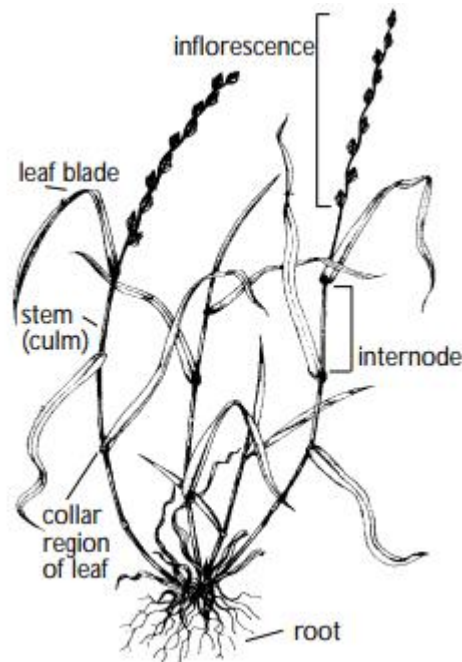


Figure 2.2: Feature of the perennial ryegrass plant sourced from Hannaway et al. (1999).

While perennial ryegrass cultivars can differ in their tolerances and growth rates in different regions, ryegrass has several defined features that allow it to be distinguished from other grasses (Figure 2.2). The ryegrass leaf blade is dark green and hairless with a ribbed upper surface and glossy underneath. The inflorescence is a spike between 5-30 cm long with a variable number of attached spikelets, each individual spikelet has between 3 and 10 florets which are the fertile part of the flowerhead. *Lolium perenne* differs from other ryegrass species as the lemmas are awnless. The collar region is narrow and hairless with small,

claw-like auricles, the ligule is also small and relatively inconspicuous and the base of the leaf sheaf can be a purplish red colour. The reproductive stems of ryegrass are divided by nodes and internodes with a leaf coming out of each node, these stems can reach between 30 to 100 cm in height (Hannaway et al., 1999; Langer, 1977). In contrast, the vegetative stem of the ryegrass plant is short with little elongation of the internode, this means the stem and node is below general grazing height and can continue to produce new leaves and tillers after defoliation (Hunt & Field, 1978).

### 2.2.2 Ryegrass Cultivars

There are several different cultivars and endophyte combinations that have become available in New Zealand since ryegrass was first introduced. The timing of the reproductive cycle in ryegrass can vary with cultivar types which are distinguished by heading date. Heading date for a cultivar refers to the time when 50% of seed heads have emerged, 22nd October is often referred to as day 0/mid-season heading date with early headings dates ranging up to 17 days before and late heading dates up to 25 days after (Lee et al. 2012).



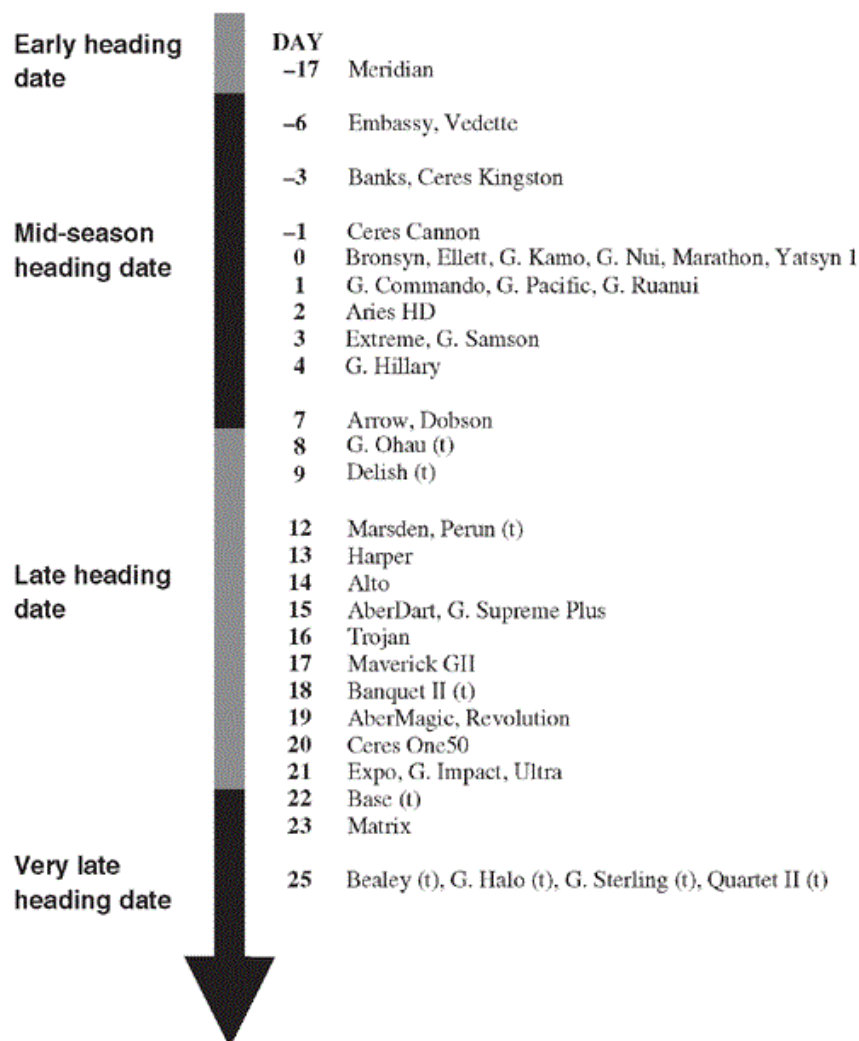


Figure 2.3: Heading dates of New Zealand ryegrass cultivars. List of cultivars and heading dates with day 0 referring to 22 October, sourced from Lee et al. (2012).

Another way ryegrass cultivars are classified is by their chromosome number, cultivars can be diploid or tetraploid. Originally perennial ryegrass was a diploid plant with 14 chromosomes, plant breeding has resulted in tetraploid plants which have double the number of chromosomes per cell. Tetraploid cultivars generally have bigger cells allowing for greater water content and larger leaves and tillers (Charlton & Stewart, 1999).

### 2.2.3 Endophytes

Perennial ryegrass can become infected with a type of symbiotic fungus that lives endophytically within the plant tissues. Fungal endophytes can produce toxic alkaloids which have been associated with staggers in livestock that have grazed down to the sheath material on ryegrass pasture. Ryegrass staggers is a disease

which causes muscle tremors as well as affecting animal growth and depressing hormone levels. Affected stock are less productive and are at risk of death in severe cases (Prestidge, 1993). The fungus spreads from infected plants to seeds and can remain viable in stored seeds for several years (Latch & Christensen, 1982). Endophyte containing pastures and the incidence of ryegrass staggers were a large concern in New Zealand prior to the introduction of novel endophytes which were less toxic to livestock (Milne, 2006)

The endophytic fungi associated with perennial ryegrass have been extensively studied over the years due to the impact on livestock as well as the implications of an endophyte infection for ryegrass persistence. Earlier studies comparing endophyte infected strains with endophyte free strains found that infected perennial ryegrass had higher dry matter yield, larger leaves and greater tiller numbers compared to endophyte free plants (Latch et al., 1985). More recent studies have recognized the importance of endophyte infection for pest management. Endophyte presence aids ryegrass's ability to resist damage caused by pasture pests, including the African black beetle which feed on plant roots (Karpyn Esqueda et al., 2017). Different strains of endophytes have also been tested for improved resistance against the Argentine stem weevil, which is one of the first introduced and most widespread pasture pests in New Zealand (Ruppert et al., 2017)

AR37 and AR1 are two examples of commercially available endophytes currently in use in the New Zealand pasture system, and are distinguished from each other based on their differing alkaloid profiles (Moate et al., 2012; Popay & Gerard, 2007). These endophytes have characteristics that reduce the occurrence of staggers in livestock over wild type endophytes, while still improving persistence of ryegrass (Fletcher & Sutherland, 2009). AR37 in particular has been proven to result in reduced damage against the larval stage of the endemic pasture pest *Wiseana cervinata* as well as *Aploneura lentisci* (Jensen & Popay, 2004; Popay et al., 2012). Studies have also found ryegrass infected with AR37 in the North Island to be higher yielding in dry matter over ryegrass infected with other endophytes (Hume et al., 2007). The relationship between endophyte type and pest resistance makes endophyte presence another important factor in improving productivity and persistence of ryegrass, with benefits gained by using a cultivar

and endophyte combination based on climate and what pests are highest risk (Popay & Hume, 2011).

#### 2.2.4 Seasonal production

The variety of available ryegrass cultivars and endophytes has helped with the continued widespread use of ryegrass in New Zealand pastures. As a perennial, ryegrass has a longer lifespan than common annual grasses and is capable of producing many tillers (Langer, 1977). The tillering ability of ryegrass allows for its good establishment and high dry matter yields. Perennial ryegrass also has high digestibility ratings of 75-85% that have made it a preferred grass species for animal production (White & Hodgson, 2005).

Perennial ryegrass growth is mostly dependent on climatic conditions and while it grows well in much of the New Zealand climate, ryegrass struggles with temperature extremes and will become dormant in hot or drought conditions (Thorogood, 2003). Certain cultivars or associated endophytes will have better tolerances for specific limiting factors which can cause variations in seasonal growth patterns. In winter, the main limiting factor for ryegrass is low temperature. Breeding has resulted in growth rates that can vary from 5-25 kg/DM a day in winter (Stewart, 2014). However, comparison between perennial ryegrass cultivars shows yield over winter months is usually much lower than in other months (Easton et al. 2001).

Dry matter production for ryegrass cultivars has been found to be highest in spring and autumn, depending on rainfall. However, differences in cultivars will be seen typically as a result of how the cultivar fared during the previous season, or due to heading date in spring (Langer 1997; Stewart, 2014). Besides showing that higher dry matter yields are produced at the time of heading, studies in Northern Ireland have also found that heading date impacts timing of tiller turnover and density in spring and early summer (Laidlaw, 2004; Laidlaw, 2005).

In summer, moisture and heat stress become the largest limiting factors for ryegrass production (Nie & Norton, 2009). Comparison amongst old New Zealand cultivars have found regional differences in performance between islands and the biggest range in production occurs particularly in summer (Easton et al. 1997).

Research conducted by Brougham (1959) supports the importance of local micro-climate for pasture production. This work found that in well irrigated ryegrass pasture, dry matter yield could be an average of 136 kg/hectare in early summer, compared to 11 kg/hectare in winter. However weekly variations in growth could be up to 50% between weeks due to fluctuation in weather, light availability and temperature. Another study that compared the growth of a ryegrass based pasture in the Waikato and Bay of Plenty regions also found differences in seasonal growth rates with peaks in growth happening at different times of the year, although the effect was in part attributed to competition with other grass species in the pasture decreasing the presence of ryegrass and not to climate differences alone (Baars et al., 1991). Ryegrass has the potential to provide high dry matter yields in summer as long as heat and moisture stresses are not severe.

Research has been done into the effect of both drought and temperature on ryegrass. One study exposed ryegrass to 30-35°C for over a month and to combined heat and drought stress (Jiang & Huang, 2001). It was found that both heat and combined heat and moisture stress affected ryegrass, and that the combined stress was more severe. Effects of heat were a decline in photosynthetic rate, leaf photochemical efficiency, and an increase in electrolyte leakage. The heat that the ryegrass was exposed to in that study is possible under glasshouse conditions, but was higher than the typical temperature ryegrass will be exposed in New Zealand summer pasture.

### **2.3 Above-ground plant response to grazing activity**

Plants under grazing pressure may not respond consistently to defoliation. There are two possible responses that may occur in a plant. A negative response occurs when growth after defoliation is less than the biomass that was removed or damage has occurred. A positive response to defoliation is often termed compensatory growth and occurs when plants produce more biomass than was removed after removal of top biomass, allowing for recovery after grazing (Ferraro & Oosterheld, 2002). Besides plant stresses that can limit growth after defoliation, other ways grazing affects plant responses can be determined by the type of stock used to graze a pasture. Different types of stock will graze more or less selectively on different species and plant parts, for example, cattle are more

likely to eat both the leaf and stem of a plant, and consume more dead material than sheep. Intensity of grazing can further affect pasture response through treading or urine inputs with different pasture species being more or less vulnerable to such interactions. For example, clover is more susceptible to treading damage than most grasses (Matches, 1992).

### 2.3.1 Ryegrass Tiller Dynamics

In perennial ryegrass, tiller dynamics will follow seasonal patterns as well as defoliation-based influences. Different cultivars can have different tillering responses to grazing which may further affect competitive ability in pasture (Gautier et al., 1999). The addition of N fertilizer also tends to positively influence tiller numbers outside of seasonal variation (Bahmani et al., 2003). However, the main period over which grazing may influence tiller dynamics is grazing in the period of reproductive development, where hard grazing of pasture interrupts elongation of tillers and reduces seed head emergence (Korte et al., 1984).

Ryegrass tillers go through a cycle of birth and death. The birth rate and survival of new tillers is an important part of maintaining stable populations within a pasture (Matthew & Hamilton, 2011). Over the reproductive period of ryegrass, about 50% of tillers may become reproductive while the rest remain vegetative. Reproductive tillers are overall more likely to die than vegetative tillers over spring, and tend to be susceptible to dying following decapitation. The death rate of vegetative tillers is higher after defoliation in summer/autumn compared to vegetative tillers in other seasons (Korte et al., 1985; Thom, 1991). However, other research has recommended allowing the development of seed heads and then hard grazing as this stimulates the development of new tillers (Xia et al., 1990).

The seasonal trends of tiller appearance and death tend to be that tiller appearance is high over summer, declines again in autumn and increases in winter while tiller death is high in summer and low in winter (Korte, 1986). The high rates of tiller appearance and death occur over the reproductive period of ryegrass, some of this is caused by a self-thinning processes that occur as competition for light between tillers increases (Edwards & Chapman, 2011).

### 2.3.2 Water soluble carbohydrates

Water soluble carbohydrate refers to the soluble carbohydrate content made up of mostly fructan as well as fructose, glucose and sucrose (McGrath, 1988). High WSC content has been correlated with both higher nutritive value as well as improved drought tolerance and regrowth after drought in perennial ryegrass (Smith et al., 2002). WSC in grasses is usually accumulated in the leaf and stubble of vegetative tillers (Lee et al., 2010). When tillers are defoliated, WSC decreases and will replenish after the emergence of new leaves. This happens as WSC reserves are mobilised from the stubble or residual leaves to support new growth. Changes in WSC to support new growth or tiller initiation is a factor in allowing ryegrass to survive under continuous grazing. Production of leaves for photosynthetic tissues is prioritised over tiller development or root growth, this can cause root or tiller development to stop under frequent defoliation (Lee et al., 2010). In reproductive tillers WSC is stored in the internodes and seed head and carbohydrate content is decreased from the leaf (Trethewey & Rolston, 2009). Reproductive tillers can also translocate carbon for the formation of new daughter tillers while plants with higher WSC reserves are more able to support the production of new seeds and tillers (Matthew, 2002). WSC reserves are important for the initiation of new tillers as daughter tillers are dependent on mature tillers for nutrients and water until they develop roots (Warringa & Kreuzer, 1996).

## 2.4 Grazing Management Strategies

Grazing management strategies include variations in the timing, stocking rate and duration over which stock have access to forage. Pasture can be grazed continuously or rotationally where the pasture gets a period of rest in between grazing cycles (Campbell, 1966; Walton et al., 1981). Continuous grazing with a set stocking rate and rotational grazing with defoliation intervals based on pasture growth are both used for hill country sheep and beef farms (Clark et al., 1982).

### 2.4.1 Timing of grazing

Methods used by farmers to assess when a pasture is ready to be grazed is through either estimation of dry matter yield or by determination of the current leaf stage. Recommended strategies for grazing ryegrass is at the 2.5-3 leaf stage (Fulkerson & Donaghy, 2001). This is because ryegrass tillers typically only retain 3 green

leaves at a time. When new leaf initiation occurs, the oldest leaf, which is usually the fourth leaf, will senesce and die. In an intensively grazed pasture system, this dying of the oldest leaf is considered wastage, therefore optimised grazing occurs before this happens at approximately the 3 leaf stage (Fulkerson & Donaghy, 2001). However, grazing too close to the 2 leaf stage or earlier can reduce pasture regrowth. Grazing based on dry matter production of a paddock is recommended between 2600-3200 kg/DM/ha. Grazing at lower or higher levels of dry matter can negatively impact either the pasture or animal production in terms of growth or milk yield (McCarthy et al., 2014).

#### 2.4.2 Deferred Grazing

Deferred grazing described for use on New Zealand farms is a variation on rotational grazing methods where a pasture is removed from the grazing rotation in spring and not grazed again until later in summer or autumn. Since ryegrass is a target species, the timing of deferred grazing is recommended according to the timing of the ryegrass reproductive cycle, with paddocks being deferred from tiller elongation in mid-October/mid-November until seed maturation in mid-January. After the closure period, standard rotational grazing is resumed (McCallum et al., 1991). This closure period is necessary for ryegrass to go through the reproductive cycle because hard grazing will interrupt reproductive development, with the result that the pasture remains largely vegetative.

Encouraging vegetative growth of ryegrass in spring is relevant in situations where maintaining feed quality is of importance, as reproductive tillers have lower nutritive value than vegetative tillers (King et al., 2016). However, benefits have been recognised in allowing reproductive development of ryegrass for encouraging the formation of daughter tillers (Matthew et al., 1991). Allowing seed maturation may further allow for natural reseeding to occur. Seedling establishment could increase tiller appearance and persistence of swards in pasture where relying on vegetative tiller propagation is insufficient (L'Huillier & Aislabie, 1988). The expectation for deferred grazing is that tiller appearance for ryegrass can be increased which will support persistence of ryegrass in pastures systems.

Other potential benefits of deferred grazing for pasture include increase dry matter and flexibility of feed supply availability (Devantier et al., 2017), as well as higher soil moisture levels and increased legume content (Harris et al., 1999; Tozer et al., 2020). Existing New Zealand literature for how deferred grazing can benefit hill country farms has focused on the above ground effects. There is currently limited research on the belowground effects of deferred grazing.

## **2.5 Pasture Roots**

Roots are an important part of a plant's ability to uptake nutrients and water from its environment. However, root morphology and the ability to uptake nutrients can vary between species. Differences in root morphology include features such as root lengths, root hair length, and root cylinder volumes (Haling et al., 2016; Yang et al., 2017). Root morphologies can differ widely in pasture species with some species having more fine and extensive root systems and other smaller and thicker. These features can usually be related to different nutrient requirements in plants (Hill et al., 2006). Root characteristics can also vary in response to soil type, water availability and competition with other plants, which can most strongly affect rooting depth and density (Nie et al., 2008; Peek et al., 2005; Remison & Snaydon, 1980). Seasonal variation and tissue turnover also occur which leads to changes in underground biomass (Saggar & Hedley, 2001). The majority of root biomass is often located in the top 200 mm, this pattern applies to several different pasture species, though cocksfoot and ryegrass have also been found with high root numbers in the top 400 mm (Evans, 1978).

### **2.5.1 Variation in roots**

There is a large amount of variation possible in root development and architecture which leads to variation in total root biomass. Roots systems can to some extent be divided into different descriptive categories, including tap roots, stoloniferous, and fibrous roots (Fry et al., 2018). While certain species are more prone to having roots in one type of category, there is a range of plasticity possible in rooting strategies, for example ryegrass tends to have a fibrous root system but can also form stoloniferous growth of new roots and tillers (Matthew et al., 1989).

Several common broadleaf species, including dock, chicory and plantain, tend to have a large, thick tap root. These species differ from each other with chicory



tending to have a larger, deeper taproot and plantain a more fibrous root. This means plantain can have a higher root density while chicory has a greater total root biomass (Cranston et al., 2016; Monaco & Cumbo, 1972).

Stoloniferous root systems are associated with various plant species including clover. Stolon growth can refer to a segment of vascular tissue that is formed below the growing point of a tiller. Stolons can grow in a lateral direction and produce roots at nodes along their length as well as initiate the formation of new tillers (Matthew et al., 1989; Stevenson & Laidlaw, 1985).

Most grass species have a fibrous roots system, these roots can be small with a high density. These root systems form with primary and secondary roots, and root hairs. Primary roots are the first roots that appear in seed germination, as secondary roots develop, the primary roots will die off. Root hairs in fibrous root systems are very fine and branch off of the secondary roots (Akkar & Mahdi, 2017).

### 2.5.2 Implications

Despite the importance of roots as a part of the whole plant system and the development of several different methods such as use of coring or rhizotrons for measuring roots, their belowground nature presents challenges for obtaining data on their distribution or biomass (Majdi, 1996). This has limited research on how root biomass is influenced during or after a cycle of deferred grazing.

When and how pastures are grazed has the potential to influence pasture composition, growth rate and dry matter yield. These are important factors for hill country farmers to consider when trying to improve pasture persistence of desirable species such as ryegrass. Previous research suggests plants will benefit from a period of deferred grazing that allows for the formation of reproductive tillers but the length of response after such a period and influence on root growth still require further investigation.

### **3 Effect of deferment length on pasture composition and root mass in an established ryegrass pasture**

#### **3.1 Introduction**

In recent years, increasing pressure has been placed on farmers to manage their grazing pastures in ways that are environmentally sustainable while still maintaining profitability. Hill country farmers in New Zealand have also had to contend with poor pasture persistence exacerbated by dry summer conditions. This is becoming an increasingly significant issue as droughts are expected to increase in frequency and severity with climate change (Booth et al., 2020). Alongside lower productivity, pastures may experience increased runoff and sediment loss into water catchments when above and below ground biomass has been reduced by poor persistence of desirable species (Bartley et al., 2014; Zhang et al., 2012). Adjusting grazing management strategies in use on farms, such as by withholding pasture from grazing between late spring and summer, has potential to improve pasture persistence by increasing the proportion of preferred pasture species and improving pasture yield.

The concept of deferred grazing allows for farm productivity to be maintained over the spring period when growth rate is high and grazing can be intensified on better performing pastures. This helps uphold vegetative growth and nutritive value (Michell et al., 1987), while lower persistence pastures can be deferred during the reproductive period as a low input method of pasture improvement. Deferring grazing over the reproductive period has been found to increase above ground herbage production and tiller numbers when grass has been withheld from grazing until seed heads are produced (Hernández-Garay et al., 1997; McCallum et al., 1991). Studies in Australia have used the timing of heavy grazing and pasture rest to increase prevalence of desirable species and decrease undesirable species (Kemp et al., 1996). An Australian based study by Dowling et al. (1996) suggested that the percentage of perennial ryegrass in a pasture could be increased by resting over the summer period. Allowing pasture to go through reproductive development could further increase pasture persistence by encouraging natural reseeding to take place (L'Huillier & Aislabie, 1988; Nie et al., 1999). In the New Zealand pasture system, perennial ryegrass is one of the most commonly sown

pasture species due to its high potential for production and nutritive value compared with other grasses (Lee et al., 2012). However ryegrass is lacking in drought tolerance and can struggle to persist in pasture, especially in summer dry conditions when it may be outcompeted by more drought tolerant species (Korte & Chu, 1983).

Evidence also suggests that greater biomass and rooting depth in perennial ryegrass could potentially improve competitiveness against other species and survival when water stressed (Hofman & Ennik, 1982; Matthew et al., 2012). Greater root biomass could further benefit pasture plants by improving uptake of nutrients (Ehdaie et al., 2010). Under constant environmental conditions, a close link between root biomass and defoliation frequency has been identified in ryegrass (Ennik & Hofman, 1983). Deferred grazing could potentially induce an increase in root biomass, however there is still little known about the extent of changes in root biomass and depth under deferred pasture. It is also unknown how the structure and dynamics of the root system are affected once grazing resumes.

Research about the above and belowground changes in pasture biomass that occur in response to deferment and standard rotational grazing will be beneficial for farmers to understand how long a pasture should be deferred for and what benefits to expect. By investigating changes in pasture composition, it will be possible to estimate which species are prevalent in aboveground dry matter and below ground biomass. Adjusting the length of the deferment period will further add to existing knowledge to improve estimates on how grazing of pasture should be timed to support the growth of perennial ryegrass.

This study took place on a hill slope in an established ryegrass pasture to quantify the effects of deferment in an environment similar to other hill country based pastures currently in use for grazing. The purpose was to test the hypothesis that aboveground pasture and belowground biomass increase when pasture is deferred in spring and that this increase will be greater under an extended period of deferment ending in autumn.

## **3.2 Materials and Methods**

### **3.2.1 Site**

The field site was located on undulating farmland at the Ruakura Research Centre in Hamilton, New Zealand. The trial was established on a north/north-west facing slope 37° 46' S, 175° 18' E with an elevation approximately 42-40 metres above sea level. The chosen site was an existing hill paddock sown on clay loam with a perennial ryegrass and white clover mix sown more than 20 years ago. Prior to the trial commencing the paddock had been routinely grazed with cattle when pasture dry matter reached between 2000 - 3000 kg DM ha<sup>-1</sup>, with grazing residuals averaging 1200 kg DM ha<sup>-1</sup>. During the trial, a herd of 30-50 cattle were given access to the plots for approximately 6-8 hours, except in May when they were given 24 hours.

### **3.2.2 Soil testing**

Soil testing was conducted by taking cores at 2 m intervals along two 50 m transects at the top third and middle third of the slope to check for aluminium levels which would be detrimental to root growth (Panda et al., 2009). Cores were taken at depth and cut into segments to obtain representative data at 0-75 mm, 75-150 mm and 150-300 mm. Results were within acceptable ranges (0.2-0.6 mg/kg). Further soil samples were taken for testing at the location of each trial plot at a depth of 75 mm. These samples were tested for standard variables including mineral content, organic matter and pH to compare between plots and replicates. Nitrogen %, organic matter and pH were all within recommended values (Table 3.1). Plots 8-10 had lower than ideal Olsen P values.

Table 3.1: Soil properties of the 12 plots at the trial site and ideal ranges recommended by Hill Laboratories (Hamilton, NZ)

Plot #	pH	Olsen P (mg/L)	Potassium (me/100g)	Calcium (me/100g))	Magnesium (me/100g))	Sodium (me/100g)	Organic Matter	Total Carbon (%)	Total Nitrogen (%)
1	6.1	34	0.63	10.6	1.23	0.19	10.1	5.9	0.49
2	6.1	24	0.46	9.8	0.93	0.18	8.9	5.2	0.47
3	6.1	25	0.38	9.2	0.92	0.2	9.3	5.4	0.44
4	6	21	0.39	8.3	0.83	0.16	8.9	5.2	0.42
5	6	23	0.44	9.6	0.86	0.17	9	5.2	0.43
6	6.1	31	0.73	10.2	1.23	0.19	9.9	5.7	0.48
7	5.9	26	0.57	9.8	1.08	0.2	8.9	5.1	0.46
8	6.2	17	0.61	8.2	0.86	0.19	8.2	4.7	0.37
9	6	16	0.41	8.6	0.9	0.21	9.3	5.4	0.44
10	5.9	14	0.53	7.6	0.93	0.18	9.4	5.4	0.4
11	6	24	0.58	7.9	0.81	0.17	9.2	5.3	0.45
12	6	30	0.92	10	1.27	0.16	11.1	6.4	0.52
Ideal Range	5.8-6.3	20-30	0.50-0.80	6.0-12.0	1.00-3.00	0.20-0.50	7.0-17.0	4.0-10.0	0.2-0.5

### 3.2.3 Endophyte infection

Endophyte infection frequency was determined by using the immunoblot assay described by Hahn et al. (2003). Fifty ryegrass tillers were taken from different plants at randomly selected locations within each plot. Sampling took place in November as the trial commenced and was repeated the following autumn. Each tiller was cut with a sharp scalpel and the base of each tiller was then firmly pressed against blotting paper which was kept in a sealed envelope in the fridge and processed within a week. Endophytes were present in the field at both dates at a high infection frequency (Table 3.2).

Table 3.2: Endophyte blot test results in late spring and the following autumn

Plot #	November-19		May-20	
	# Positive Tillers	Frequency Infection %	# Positive Tillers	Frequency Infection %
1	49	98	36	72
2	49	98	50	100
3	47	94	48	96
4	49	98	47	94
5	49	100	45	90
6	49	98	46	98
7	50	100	50	100
8	50	100	50	100
9	49	98	48	96
10	48	96	49	98
11	50	100	40	80
12	45	90	50	100

### 3.2.4 Experimental design

The trial was designed as a randomised complete block with three treatments and four replicates. The three grazing treatments included a standard rotational grazing control (Def x0) which was the current management technique in use on the farm. The second treatment was a 14 week deferred treatment (Def x14) which was excluded from grazing from early November until the end of February, allowing the ryegrass to flower and set seed. The third treatment was a 27 week deferred period (Def x27) which was excluded from grazing from early November until the middle of May. Treatment Def x0 was grazed once in December according to the amount of available dry matter and not again until the opening of Def x14 in February as growth in all plots was slowed by drought conditions. Treatments Def x0 and Def x14 then experienced a longer than usual rest period between grazing rounds, from the grazing in February to the opening of the last Def x27 treatment in May because Covid-19 pandemic restrictions resulted in researchers being prevented from accessing trial sites (Figure 3.1).

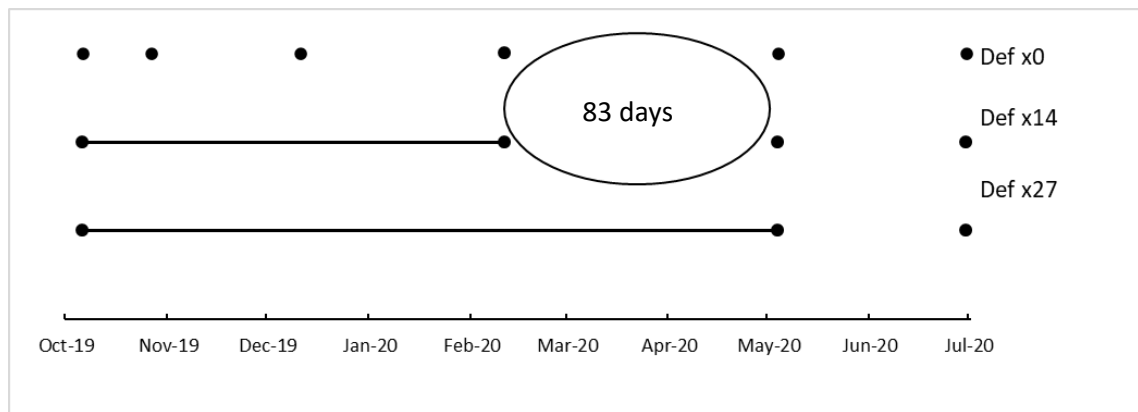


Figure 3.1: Timing of deferment treatments and grazing periods. Dots show when grazing happened and lines indicate the initial deferment periods. Def x0 was grazed at approximately 2000 kg DM ha<sup>-1</sup> except in February when dry matter was approximately 800 kg DM ha<sup>-1</sup> due to the effect of drought and bare ground, and in May when dry matter was approximately 3200 kg DM ha<sup>-1</sup> as paddocks were excluded from grazing for 83 days over the Covid-19 lockdown. Def x14 was last grazed in October at approximately 2000 kg DM ha<sup>-1</sup> and deferred from grazing until the end of February, it then wasn't grazed again until May (the 83 gap created by the lockdown period) at approximately 3200 kg DM ha<sup>-1</sup>. Def x27 was last grazed in October at approximately 2000 kg DM ha<sup>-1</sup> and deferred until the May. All treatments were grazed at approximately 2000 kg DM ha<sup>-1</sup> in July.

The field site established with six pairs of 12 x 14 m plots arranged up the slope and positioned to avoid the flattest part of the top of the hill and bottom of the slope (Figure 3.2). A three metre raceway through the middle of the slope split the plots into a set of six on either side of the slope. This allowed for cattle to comfortably move between open plots for grazing without compromising the closed off plots.

Five grazing cycles occurred during the field trial. This period included baseline measurements in November, grazing of control plots (Def x0) in December, end of the short deferred treatment (Def x14) in February, end of the long deferred treatment (Def x27) in May and a final measurement harvest 2 months later in July after normal grazing had been resumed for all treatments.

Each of the 12 plots was split into seven 2 metre sampling strips from which measurements from each harvest were taken. Two of the sampling strips were used for another trial and the last five were planned to be utilized for the measurements reported here, as well as ongoing measurements in a second year.

The purpose of the sampling strips was to avoid repeat sampling of roots in areas affected by vehicle traffic during root sampling using a pneumatic corer mounted on an LUV. All measurements taken during each harvest were taken from the same transect to build a profile of the above ground plot condition that would most closely match the area from which underground conditions were being sampled. The order of transects sampled was assigned randomly and a new transect was sampled for each harvest.

In each plot, sensors were installed to measure air temperature (DS1922L iButton temperature logger, Maxim Integrated, USA.) soil temperature and soil moisture content (Odyssey Xtrem soil moisture logger, Dataflow Systems, NZ.). The sensors were protected under a wire cage and the pasture around them cut by hand to the length of the adjacent pasture following each grazing round. Climate history data was recorded by the Ruakura Weather Station which is situated approximately 900 m from the site the trial took place.



Figure 3.2: Pre-grazing photos of one side of the trial paddock showing seasonal pasture changes. Tall fence standards mark the corners of the plots and white stakes mark the position of the sampling strips across the plots



### 3.2.5 Measurements

The timing of each grazing was decided based on the measured dry matter available in the grazed plots (averaging 2000 kg DM ha<sup>-1</sup> estimated by rising plate meter) and the reproductive stage (maturation of seed heads) for the deferred plots. Before each grazing, measurements were taken to determine dry matter, pasture composition, nutritive value, tiller densities and root mass.

A rising plate meter was used to obtain an estimate of dry matter immediately before and after grazing by taking 30 plate counts per plot. Pre-grazing mower cuts were also taken to measure dry matter from a strip six metres long and the width of the mower within each plot at a cutting height of 5 cm, determined by the settings on the mower. The same mower was used each time. The mown grass was collected and weighed in the field to obtain a fresh weight. An approximate 300 g subsample was then weighed, oven dried at 65 °C for 48 hours and weighed again to obtain a dry weight.

For pasture composition, visual ground cover assessments were taken from a 1m<sup>2</sup> quadrat at four separate points along the sampling strip for each plot. Percentage cover was estimated for the categories: ryegrass, other grass, broadleaf weed, legumes and bare ground.

Above ground pasture samples were taken using a 100 x 25 cm quadrat at five random points along a plot sampling strip. The pasture within each quadrat was cut to just above ground level with electric shears and bulked per plot. A subsample amounting to approximately 400 pieces was taken and separated into ryegrass, other grass, white clover, other clover, broadleaf weeds and dead. These samples were then oven dried at 65°C for 48 hours and weighed to get percentage weight of each category.

Nutritive value samples were subsampled from pasture composition. The samples were kept chilled and taken to the laboratory where each sample was thoroughly mixed and a 20 g subsample taken for nutritive value and immediately placed in a -20°C freezer. The nutritive value sub samples were freeze dried and ground to an even particle size using a mill grinder with a 1 mm sieve. The ground samples were sent to Hill Laboratories for forage analysis.

Tiller densities were recorded using a 15 x 10 cm quadrat from which all ryegrass tillers were cut. If ryegrass could not easily be separated from the other pasture species, the entire quadrat was cut to just above ground level. Fifteen quadrats were taken and bulked per plot. In the lab, the sample was mixed and 150 ryegrass tillers counted out randomly sampling from the mixed sample. Any non-ryegrass herbage that was sampled alongside ryegrass tillers was also set aside. A fresh weight was taken for the 150 ryegrass tillers, non-ryegrass and dead material samples. The samples were then oven dried at 65°C for 48 hours and dry weight recorded.

Root samples were taken with a 50 mm wide, 1 metre long pneumatic corer mounted on a light utility vehicle under 1000 kg (LUV) designed for off road use. Each plot was divided into 2 metre wide transects and the LUV moved down a new transect for each harvest. No sampling was undertaken in the areas of tire tread to avoid measuring vehicle related pasture damage or compaction effects. For each transect, cores were taken along a 10 metre strip at approximately 1 metre intervals. Cores were taken to a 30 cm depth and separated into 0-15 cm and 15-30 cm depths. Cores were bulked by depth for each plot. The high clay content of the soil proved challenging for coring, and the pneumatic corer could not be used during the February harvest as the lack of soil moisture had hardened the ground s. Only 0 -15 cm could be taken during the final harvest because of high soil moisture making the lower layer too tacky for the corer. Root depth was dropped as a measurement from the first measurement harvest as deeper cores were not feasible for the corer. The cores that were taken were washed using water mixed with a dispersing agent, sodium hexaphosphate to form a 5% solution (Wintermyer & Kinter, 1955). This assisted the separation of soil particles so that the roots could be removed from the clay under more gentle agitation than could be achieved with water alone. A 1 mm sieve was used to extract roots once separated and these were place in a container for a final rinse to remove all traces of soil. The roots were then oven dried at 65°C to a constant weight before weighing to obtain dry biomass.

This trial had a split plot design, statistical data was analysed using Genstat 20th edition VSN International using an analysis of variance (ANOVA).

### 3.3 Results

#### 3.3.1 Climate

The recorded temperatures for the spring/summer of 2019/2020 for Ruakura were within 0.5C of the 5-year average (Table 3.3). The 5-year average temperatures tended to be higher than the 10-year averages. However, January, March and April 2020 were cooler than the long term average temperatures. The winter months June and July 2020 were warmer than previous averages from the last five years, with the exception of 2019.

The average monthly rainfall for January and February 2020 was between 80-90% lower than both the 5 and 10 year averages recorded at Ruakura (Table 3.4). This was the lowest average rainfall recorded over a 5 year period for those months. Rainfall was also lower than average in November and December 2019. In the following autumn of 2020 (between March-May), rainfall was also 20-50% lower than average.

Table 3.3: Monthly mean air temperature (°C) recorded by the Ruakura Weather Station between 2015-2020 and the averages between 2015-2019 and 2010-2019 (NIWA).

	2015	2016	2017	2018	2019	2020	5-year average	10-year average
Jan	20.2	19.9	17.4	21.3	20.5	18.3	19.9	19.1
Feb	19.1	21.4	19.2	19.8	20.2	20.1	19.9	19.5
Mar	18.7	18.9	18.5	19.0	19.1	17.4	18.8	18.0
Apr	15.5	15.5	16.1	15.3	14.3	14.7	15.3	15.2
May	12.1	14.4	11.8	12.8	13.0	12.4	12.8	12.2
Jun	10.8	10.9	10.1	9.9	9.5	11.7	10.2	10.0
Jul	9.0	9.6	8.8	9.5	10.3	9.9	9.4	9.0
Aug	9.9	10.0	10.7	10.3	10.6		10.3	10.0
Sep	11.7	12.9	12.0	12.1	11.8		12.1	11.8
Oct	13.9	13.7	13.7	13.2	13.3		13.6	13.3
Nov	15.2	15.2	16.2	15.6			15.6	15.1
Dec	17.3	16.5	19.2	18.6	18.0		17.9	18.0

Table 3.4: Monthly rainfall (mm) recorded by the Ruakura Weather Station between 2015-2020 and the averages between 2015-2019 and 2010-2019 (NIWA).

	2015	2016	2017	2018	2019	2020	5-year average	10-year average
Jan	47.0	89.2	47.8	274.5	22.8	6.4	96.3	99.1
Feb	44.6		101.8	183.8	14.4	9.6	86.2	52.6
Mar	75.4		237.8	50.6	35.4	64.0	99.8	79.5
Apr	174.6	40.4	253.6	95.0	73.8	52.8	127.5	110.2
May	130.6	120.8		125.4	60.6	76.8	109.4	114.1
Jun	63.4	140.0	54.2	115.0	68.8	96.2	88.3	129.1
Jul	108.0	133.0	157.5	116.4	113.0	94.0	125.6	113.3
Aug	114.8	79.6	121.8	138.6	133.0		117.6	104.4
Sep	97.2	114.0	169.0	53.0	151.0		116.8	114.1
Oct	38.8	98.6	112.2	53.4	66.0		73.8	74.0
Nov	106.6	83.8	35.6	78.6	57.6		72.4	62.9
Dec	30.2	45.4	86.4	184.4	74.8		84.2	104.8

While the trial occurred on pre-existing pasture which had been subject to all prior climatic variables for the area over a 20 year period, the trial period itself encompassed the drought over summer and recovery over the warmer than average winter period. The warmest temperatures measured within the trial plots were recorded in February, with a maximum of 31.1 °C; the minimum temperature was -3.6 °C in July (Figure 3.3.a.). Soil moisture and precipitation show similar trends with soil moisture decreasing over the summer period and increasing under the higher rainfall from the end of February onwards. Changes in soil moisture occurred gradually in response to changes in precipitation (Figure 3.3.b). Soil moisture required from the end of February until the start of June to return to levels seen in November. January and February were both extremely dry months, no rainfall was recorded for 23 and 24 days respectively (Figure 3.3.c.). While the preceding spring and following autumn saw more days with larger amount of rainfall, there was no rain for 20 days in November, March and May. The highest peak rainfall was 30.8 mm in May while June was the wettest month with rain recorded on 19 days.

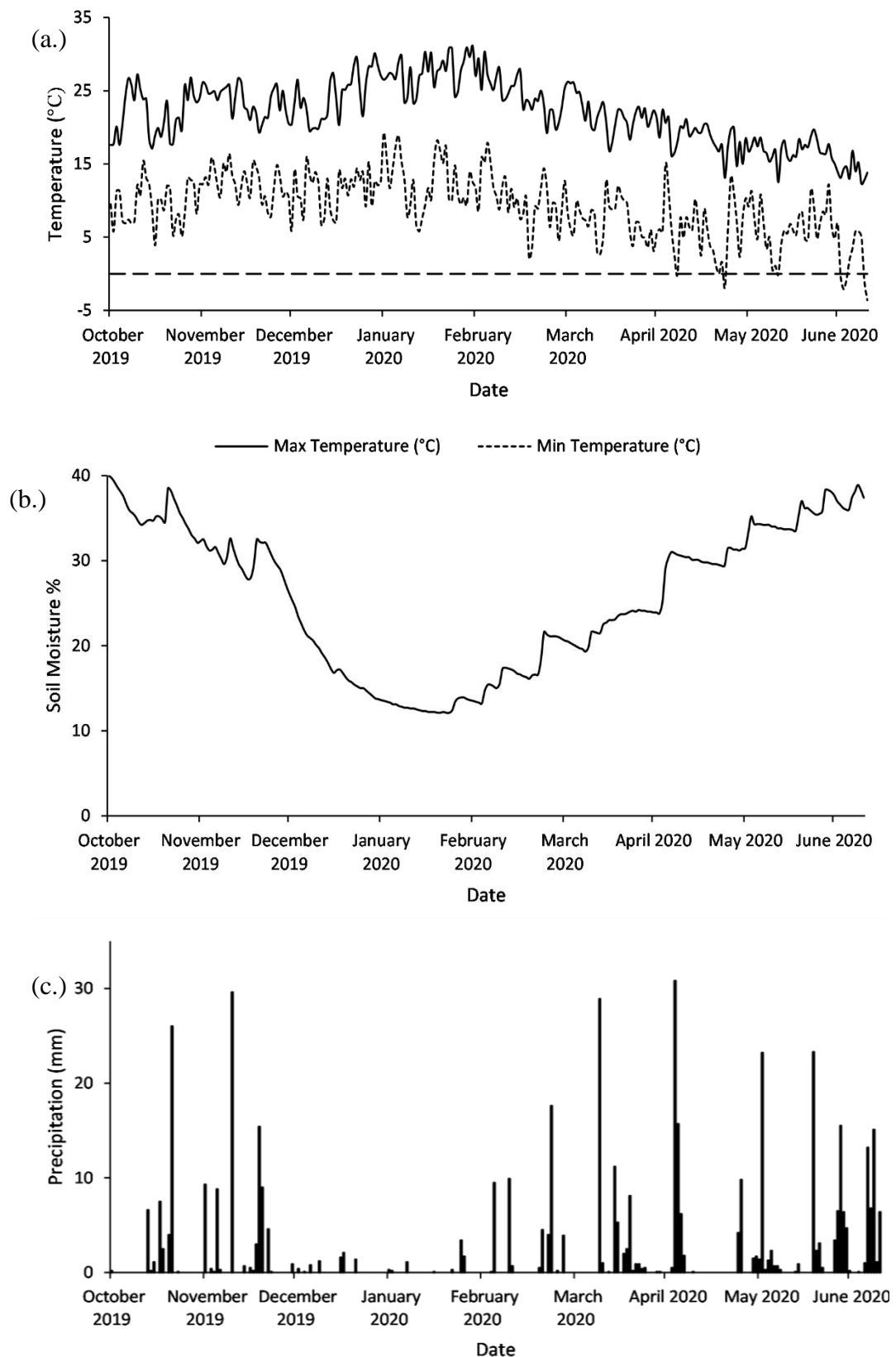


Figure 3.3: The daily changes in climatic variables most likely to influence pasture growth over the course of the trial from October 2019-June 2020. (a.) temperature range, (b.) soil moisture and (c.) precipitation. Data recorded by the Ruakura Weather Station.

### 3.3.2 Dry Matter accumulation

There was no difference between treatments in the dry matter accumulated between 10-31 October which averaged 510 kg DM ha<sup>-1</sup> and 1-30 November which averaged 670 kg DM ha<sup>-1</sup> (P>0.05).

In summer, more dry matter accumulated in Def x14 than Def x0, with Def x27 intermediate but not differing from the other two treatments (P=0.017; Table 3.5).

In autumn, more dry matter accumulated in Def x27 than Def x0 and Def x14 (P=0.002).

In February the deferred plots had four to six times the amount of dry matter present than in the control (P=0.025). In May, Def x27 had at least 1200 kg DM ha<sup>-1</sup> more dry matter present than in the other two treatments (P=0.039). In July, there were no differences in dry matter (P=>0.05).

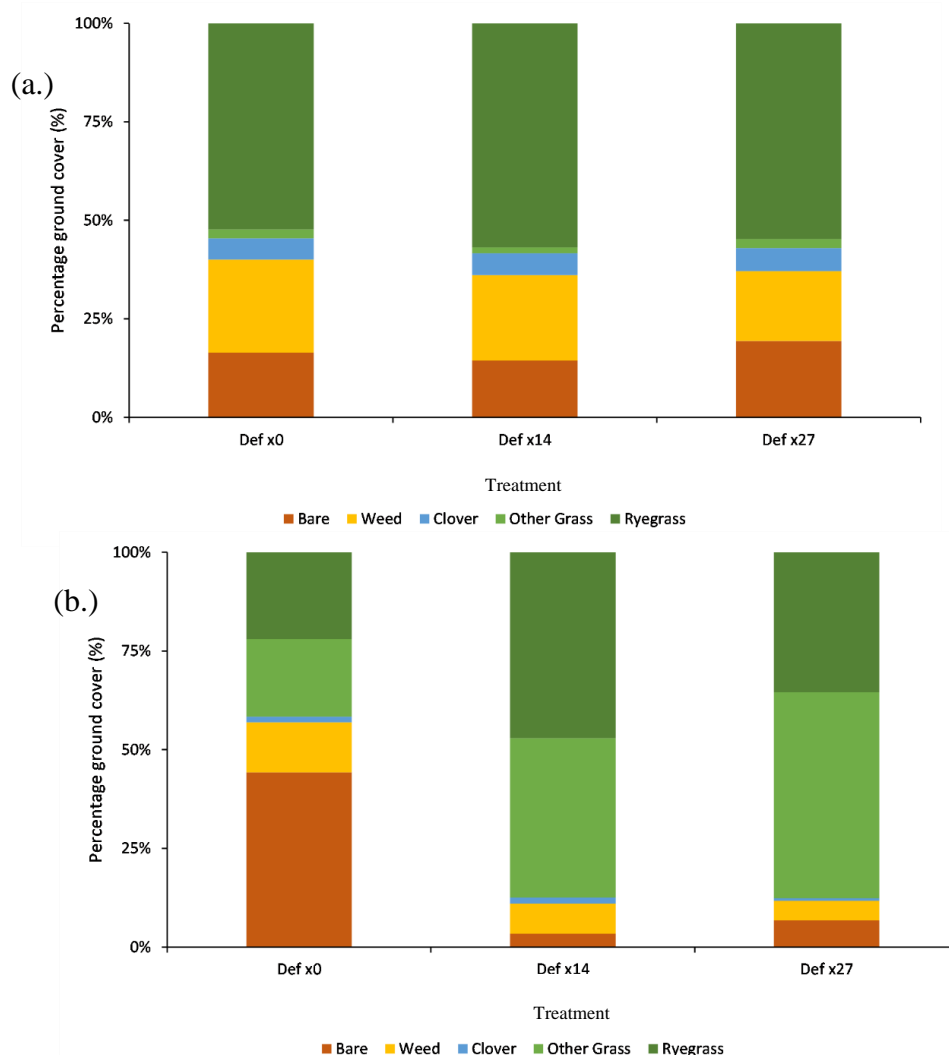
Table 3.5: Above ground pasture dry matter (kg DM ha<sup>-1</sup>) accumulated over summer (Growth from November -February) and autumn (Growth from February-May) for Def x0 (rotationally grazed), Def x14 (deferred from grazing October to February) and Def x27 (deferred from grazing October-May) and pre-grazing total dry matter (kg DM ha<sup>-1</sup>) at the end of the deferment periods (20<sup>th</sup> Feb Def x14; 13<sup>th</sup> May Def x27). Accumulated dry matter was the increase in pasture dry matter over the stated period, and pre-grazing dry matter was the total above ground dry matter present prior to grazing.

	Def x0	Def x14	Def x27	LSD	P value
Summer	830	2330	1510	888	0.017
Autumn	1110	1220	2400	544	0.002
Pre-grazing dry matter -					
February	447	2845	1843	1071.3	0.005
Pre-grazing dry matter - May	1077	1228	2474	423.3	<.001

### 3.3.3 Ground cover

Percentage ground cover of pasture varied more with season than between treatments (Figure 3.4). The largest treatment difference was in percentage bare ground during February, over 40% of the control was bare ground. In comparison, deferred treatments had less percentage bare ground than the control in February ( $P<0.001$ ). Seasonal changes in ground cover included higher percentage ryegrass early on, with other grasses increasing in percentage cover over the drought period. By May, other grasses had decreased in percentage cover and broadleaf weeds were dominant with no bare ground. In July, percentage cover was more even between pasture species types, and bare ground was present in all treatments.

Botanical dissections of the above-ground pasture samples also saw large changes in composition from season to season but no differences between treatments, except for the percentage of dead material (Figure 3.5). From May to July, Def x27 had higher percentage dead than the other treatments ( $P<0.05$ ).



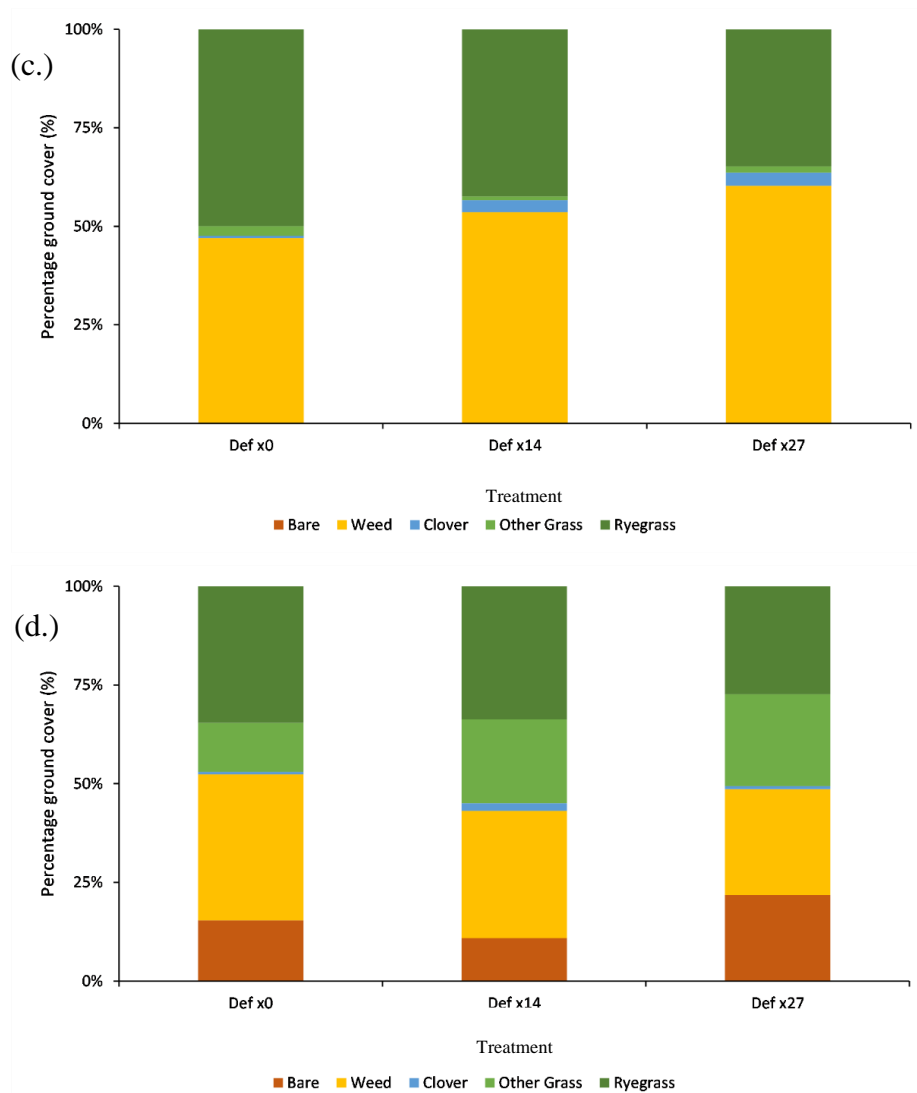


Figure 3.4: Effect of a period of withheld grazing on percentage ground cover of pasture species and bare ground. Plots were grazed with cattle at an estimated 2000 kg DM ha<sup>-1</sup> (Def x0), or after a withholding period of 14 weeks (Def x14) or 27 weeks (Def x27). Categories for percentage ground cover split into ryegrass, other grass, legumes, broadleaf weeds and bare ground over the course of the trial. (a) November, (b) February, (c) May, (d) July.



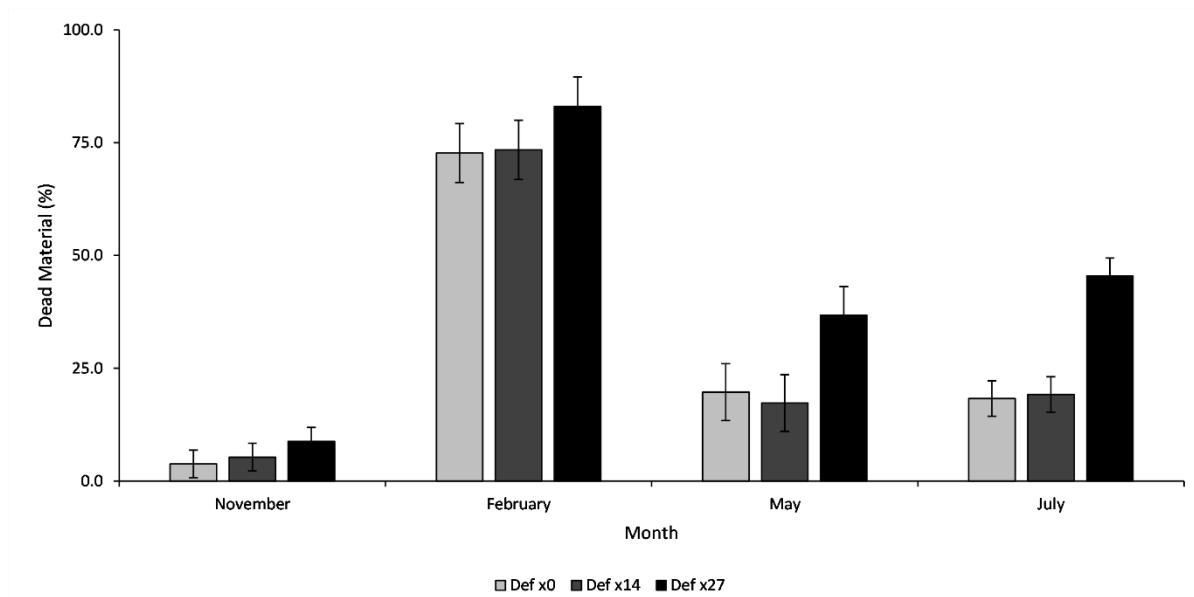


Figure 3.5: Effect of a period of withheld grazing on percentage dead material in total dry matter. Plots were grazed with cattle when there was approximately 2000 kg DM ha<sup>-1</sup> (Def x0), or after a withholding period of 14 weeks (Def x14) or 27 weeks (Def x27). Standard error bars shown (ANOVA).

### 3.3.4 Tiller densities

There were no differences in tiller densities between November and February for all treatments (Figure 3.6). After the change in season from summer to autumn and the long non-grazing period that preceded the May sampling, all treatments increased in tiller density. Tiller densities in May were higher in the control and Def x14 compared with Def x27 ( $P=0.007$ ). When normal rotational grazing had resumed in all treatments by July, tiller densities decreased to similar values compared to earlier in the year and were lower in the control than both the deferred treatments ( $P<0.05$ ).

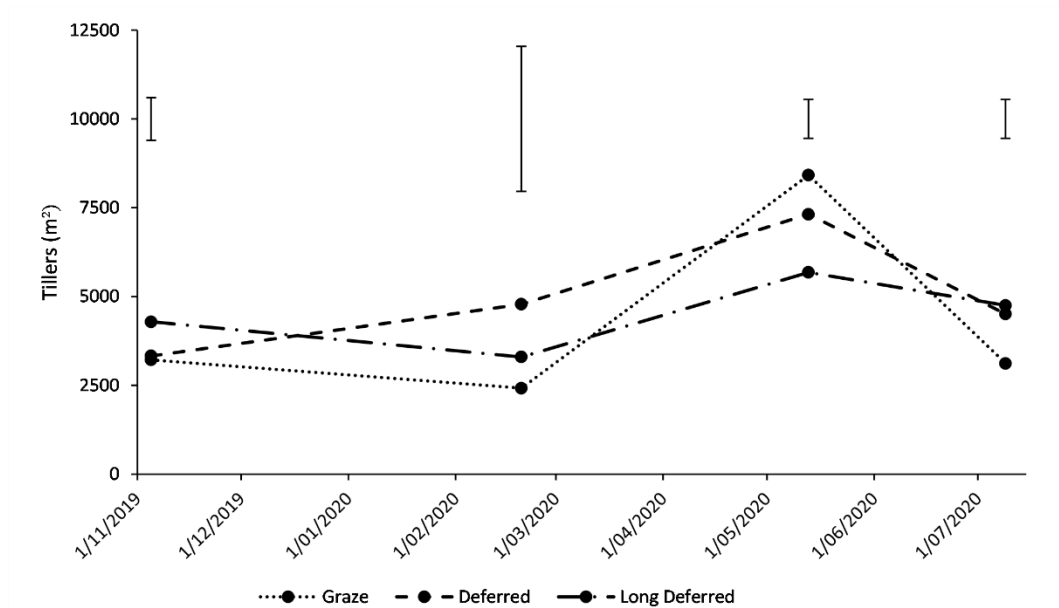


Figure 3.6: Effect of a period of withheld grazing on tiller densities. Plots were grazed with cattle at approximately 2000 kg DM ha<sup>-1</sup> (Def x0), or after a withholding period of 14 weeks (Def x14) or 27 weeks (Def x27). Standard error bars shown (ANOVA).

### 3.3.5 Nutritive value

ME for all treatments decreased from November to February and increased again in May (Figure 3.7. a.). Def x27 had lower ME in May compared with the other treatments ( $P=0.027$ ). NDF increased between November and February with no differences between treatments (Figure 3.7. b.). By May NDF was 5 percentage points higher in Def x27 than the other treatments ( $P=0.044$ ). The control treatments had higher CP than the deferred treatments in February (Figure 3.7. c.;  $P=0.004$ ). In May CP was highest in the control, intermediate for Def x14 and lowest for Def x27 ( $P=0.010$ ). All treatments had returned to similar value for the three indicators by July ( $P>0.05$ ).

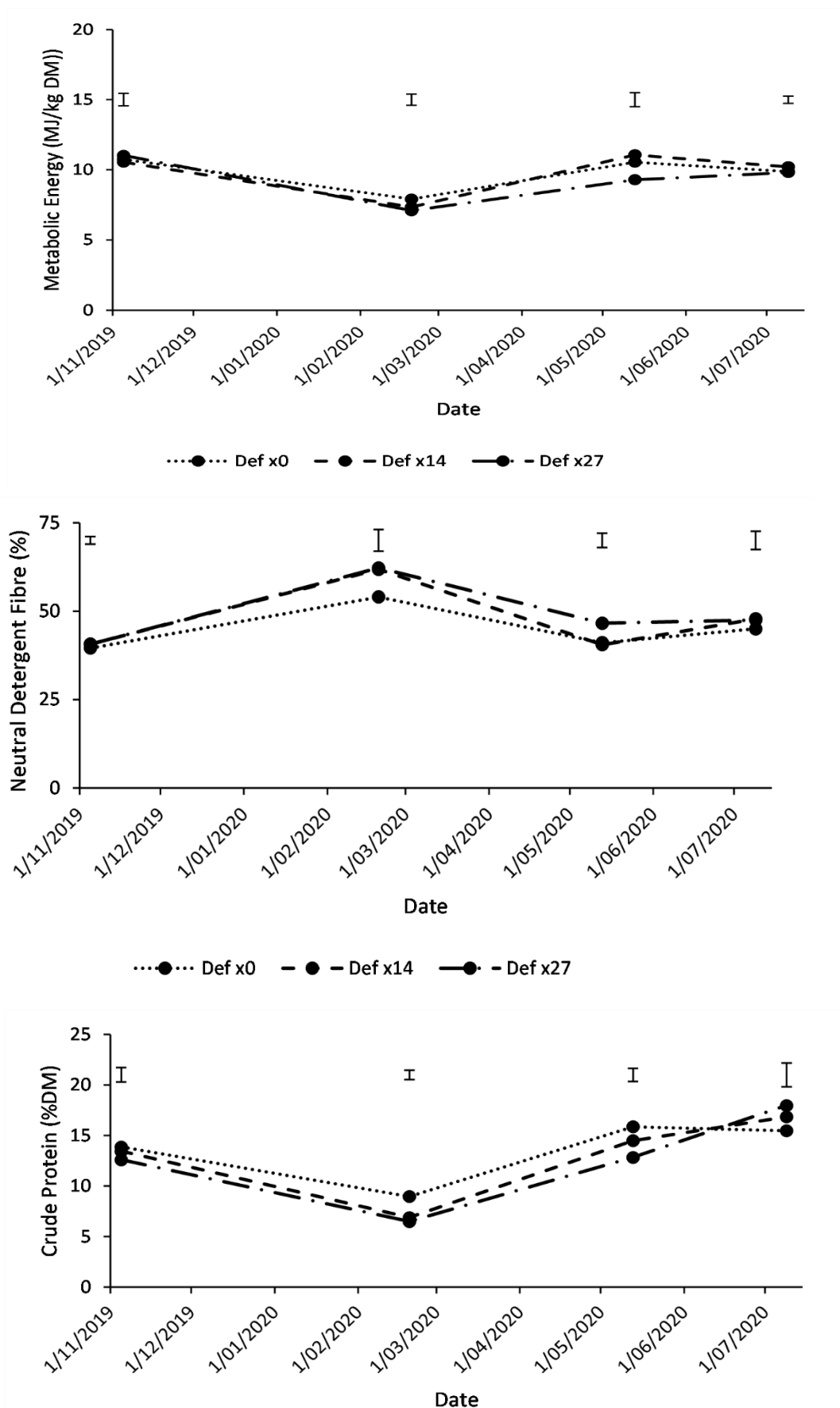


Figure 3.7: Effect of a period of withheld grazing on three nutritive value indicators. a.). metabolic energy b.). neutral detergent fibre and c.). crude protein. Plots were grazed with cattle at approximately 2000 kg DM ha<sup>-1</sup> (Def x0), or after a withholding period of 14 weeks (Def x14) or 27 weeks (Def x27). Standard error bars shown (ANOVA).

### 3.3.6 Root biomass

There were no statistically significant differences between treatments or detectable changes over time in root biomass throughout the course of the trial (Figure 3.8;  $P > 0.05$ ). The root biomass for cores taken at 150-300 mm made up between 8-12% of the biomass with the rest being located in the top 0-150 mm core. There was no treatment difference in mass when coring was possible to 150-300mm. Visual observation was made of roots down to 1 metre when coring to depth was possible, however these roots were very fine and total biomass below 300 mm was low for all treatments.

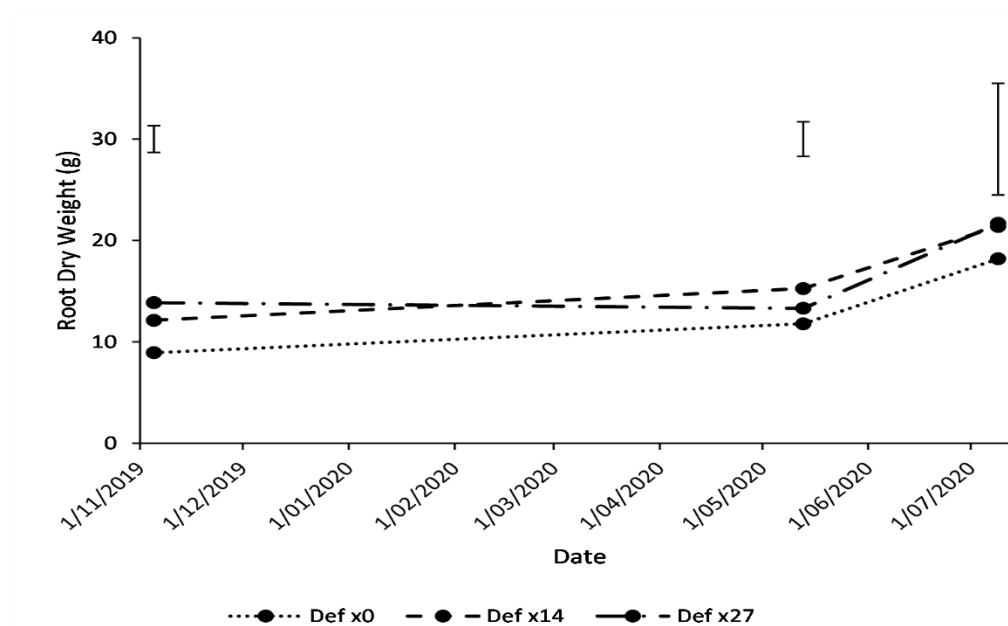


Figure 3.8: Effect of a period of withheld grazing on root biomass averaged from 10 cores taken at 0-150 mm in depth from soil surface. Plots were grazed with cattle at approximately 2000 kg DM ha<sup>-1</sup> (Def x0), or after a withholding period of 14 weeks (Def x14) or 27 weeks (Def x27). Standard error bars shown (ANOVA).

## 3.4 Discussion

When Def x14 was opened up for grazing in February, there was a greater accumulation of dry matter, less bare ground and lower crude protein, compared to the control. Crude protein also remained lower in May and tiller densities higher in July than the control treatment.

When deferred between October and May, pastures had a low amount of bare ground at the end of summer. During autumn a high amount of dry matter was

accumulated comprising of a high proportion of dead material of low nutritive value (low CP and high NDF). Tiller densities were also higher than the control in July

The hypothesis that above and below ground biomass would increase over an extended period of deferment was not supported. No changes were found in total root biomass by this study between treatments or seasons, this contrasts with the results of other studies which indicate both seasonal changes in root biomass and responses to defoliation (Caradus & Evans, 1977; Vinther, 2006). The results found in other studies suggest the sampling method used for this trial was insufficient to measure changes, rather than the root biomass not changing under deferred grazing. The sampling achieved was limited by soil type, drought and pandemic restrictions. Ideally samples should have been taken to a greater depth and with more frequency to be confident of detecting changes, or lack thereof.

Some of the largest changes seen during treatment application appeared to be seasonal or climate driven. Rainfall and soil moisture were unusually low between December and February and by the February sampling period, the trial plots were all affected by drought stress. Previous studies on the impacts of drought in ryegrass have found decreased tiller densities during the period of stress with compensatory growth resulting in a high accumulation rate after drought has ended (Barker et al., 1985; He et al., 2017). This effect, described in Korte and Chu (1983), could explain the steep increase in tiller densities found in May. Their observed compensatory growth period also lasted for about 6 weeks which aligns with the results of this study where tiller densities decreased again after grazing. Def x0 was the most drought affected treatment based on percentage of bare ground and also experienced the largest difference in tiller densities between increasing in May and decreasing in July. Under normal conditions, there would be a decrease in tiller densities for treatments left to go reproductive, and higher densities with grazing (Korte, 1986). However, this pattern was not observed in this trial with neither deferred plots showing a significant decrease from November to February. Considering the low yield from grazed plots in February and the decrease in percentage of ryegrass seen in ground cover assessments for all treatments, it seems likely that the drought restricted the expected increase in tillering caused by grazing.

While some studies have reported positive impacts on tillers by grazing during drought (Thomas & Evans, 1990), other studies have found more frequent defoliation during summer to decrease ryegrass density and persistence due to loss of water soluble carbohydrate reserves (Turner et al., 2006a). More recent studies in ryegrass persistence have also confirmed that drought related mortality is the main cause of lack of persistence (Woodward et al., 2020). It is interesting for this field study, that while the grazed treatment appeared severely impacted by the drought in February, the treatment did recover tiller densities and percent ground cover after the rest period to the same or slightly higher levels compared to the deferred treatments by May. It is unknown to what extent the recovery of the grazed treatment was affected by the longer than usual grazing rest interval and what the longer term effects of deferment on tiller densities are as the trial ended after this change in tiller densities was found.

All treatments were impacted by weed ingress in autumn with over 50% of ground cover being taken up by broadleaf weeds, of which plantain was the most common. Previous literature on grazing management has recommended the use of deferred grazing for pasture persistence as it encouraged reseeding and improved grass percentages (L'Huillier & Aislabie, 1988; Nie et al., 1999). In a study using deferring from November to January, seedling densities were 20-50 times higher than spring grazed pasture (L'Huillier & Aislabie, 1988). A separate study deferring pasture from spring to autumn in summer wet conditions found increased seed populations which favoured grasses compared with grazed pasture (Nie et al, 1999). Grass seed germination started from December through March while weed germination started from February . No seedling effect was observed for either deferred plots for this trial, however the timing of grass germination described by Nie (1999) coincided with the drought period. The self-thinning effect of reproductive ryegrass (L'Huillier & Aislabie, 1988) in deferred treatments, or bare ground in the control, could have reduced the competitiveness of ryegrass allowing for the drought tolerant weed establishment seen in all treatments in February. Nie (1999) further recommended grazing in March when rainfall was low as seedling density decreases from March until May. Based on this previous research and the results seen in this trial, it is likely the combination of drought and the unexpectedly long grazing cycle from February to May

prevented the potential for ryegrass seedling establishment in the deferred treatments. The results of this study suggest that deferred grazing will not significantly change the composition of ryegrass pastures if growth is restricted by drought or timing of grazing. It can further be inferred that the seedbank and climate conditions are important driving factors in pasture composition (Espigares & Peco, 1993; Robertson, 2006).

Overall, the treatment deferred until February accumulated more dry matter than the control in summer, and the treatment deferred until May accumulated more dry matter over autumn than the other two treatments. However, while Def x27 had greater dry matter, approximately half of it was dead material, this was significantly more than the dead material in the other two treatments. The other treatments weren't grazed as planned, therefore a fair comparison in accumulated dry matter cannot be made for autumn between the longer deferred period and the control. What can be considered is the overall use and quality of the feed supply created by the deferred opening for grazing. High total dry matter does not necessarily mean it is of high value as feed for stock. Feed quality is a measure of both intake and nutritive value (Waghorn & Clark, 2004).

Nutritive value was affected by grazing management in this trial. Reproductive growth can lessen nutritive value of feed compared with vegetative growth and a drop in metabolic energy and crude protein was expected for the first grazing after deferment (Čop et al., 2009; Turner et al., 2006b). However, nutritive value decreased in all treatments and ME was the same for all treatments in February. This trend is found in other research where nutritive value of pasture decreased during drought (Deléglise et al., 2015) and suggests the difference in nutritive value between the control and deferred at this time was lessened due to the large amount of dead material in all treatments. Crude protein increased in all treatments after February, this trend is found as a seasonal effect in other research (Weller & Cooper, 2001).

Def x27 remained lower for ME and CP in May than other treatments. Comparing the deferred treatments with other research ME for reproductive ryegrass was lower in February in this trial but similar for May through July (Fulkerson et al., 1998). In this trial all treatments had the same nutritive value by

July. This suggests that the nutritive value of the accumulated dry matter will be lower in deferred plots, and that the nutritive value will recover after grazing.

The deferred treatment opening in February meant there was a supply of feed during the severe drought period, and the deferred plots were in better condition to be grazed than the control which had not recovered fully from a December grazing. However, the lower nutritive value means the accumulated dry matter won't be as beneficial in conditions where pasture growth is not limited on the rest of the farm, such as during a wet year. Other research has recommended the use of autumn deferment for generating extra feed over winter months when growth rate decreases (Brown, 1976). It is also worth noting that higher dry matter in autumn does not mean ryegrass has benefited from deferment. A comparison of dry matter yields between Bay of Plenty and Waikato regions did find a peak in growth for autumn, though timing varied with the different regions and climates, however the increase in dry matter yield was mostly made up of other pasture species and not ryegrass (Baars et al. 1991).

The results of this trial open up some key areas for future research including questions about the use of deferment for encouraging seedling establishment or changes in the seedbank. An unknown raised by this trial that could be investigated is the effect of the long rest period after drought breaking and how longer rotational grazing affects pastures over non-reproductive periods. Finally, this trial did not answer its intended question about changes in root biomass under deferred grazing. It would be ideal to revisit root changes in response to grazing management in pasture with a soil type better suited to coring and root washing.

### **3.5 Conclusion**

Overall, the results for this trial found a period of deferment did allow for higher accumulation of dry matter compared with standard rotational grazing. However, it is not known how the effect of deferment would have changed if the Covid-19 restrictions had not prevented the shorter deferred period from retaining standard grazing cycle between February and May. Deferral of pasture between spring and summer created a feed supply during a drought period with the same ME value as the control. Deferral of pasture between spring and autumn allowed for a high accumulation of dry matter, but with low nutritive value. Both deferment



treatments had higher tiller densities going into winter compared with the control, but this effect was not well explored due to the trial ending. Deferred pasture had less bare ground during the drought period in summer but no other differences in pasture composition were evident in the following months. These results suggest a period of deferment may be beneficial in terms of accumulated dry matter and potentially offer a useful feed supply when feed is scarce elsewhere on the farm. However, the low nutritive value means overall pasture production is not necessarily improved by the accumulated dry matter. Deferring offered no clear benefit in terms of ryegrass persistence or root biomass for pasture in this trial. This suggests deferred grazing will not improve the pasture persistence of ryegrass on hill country farms when subjected to the conditions presented in this trial with a long drought period and inconsistent grazing rotation. However, further research is needed on the influence of deferment on pasture composition as well as the timing of grazing to support ryegrass seedling establishment and on belowground biomass under soil conditions better suited for retrieving cores.

## **4 Changes in above and below ground biomass of perennial ryegrass during and after different lengths of deferment under controlled glasshouse conditions.**

### **4.1 Introduction**

Perennial ryegrass is an important forage species for New Zealand pasture based farms and continues to be the predominantly sown grass on both dairy and sheep and beef farms due to its high productivity (Lee et al., 2012). Economically, the productivity of animals and the quality of the available forage is closely linked and this puts pressure on farmers to efficiently manage pastures in order to keep outputs high (Chapman et al., 2016). Further pressure is also put on farmers to use more sustainable methods due in part to increasing environmental awareness in New Zealand alongside the concerns and impacts of a changing climate (Pearson, 2020).

A major limitation of ryegrass identified by farmers has been poor persistence in pasture, this is particularly a problem in the upper North Island of New Zealand (Dodd et al., 2018). Factors that have been identified as causing lack of persistence include soil fertility as well as a decrease in the formation of new tillers or increase in death rate during drought conditions (Clark, 2011).

Understanding the interactions between grazing pressures and plants growth allows for grazing management to be adjusted to support pasture longevity and productivity.

Previous research has found benefits when grazing is withheld over the late spring and early summer period to improve pasture yield (Harris et al., 1999; Nie & Zollinger, 2012). An expected effect of using the reproductive period of ryegrass as a guide for deferment is to increase the production of daughter tillers from adult tillers and maturation of reproductive seed heads for seedling recruitment later in the year (Devantier et al., 2017; Matthew et al., 1991; McCallum et al., 1991).

This work suggests deferred grazing is a low input and sustainable way to control the renewal of pasture over the summer period when feed is usually at a surplus.

A gap existing in the current literature is how long the benefits of deferring pasture persist once grazing is resumed. While field studies have found promising

results in terms of above ground changes that remain after grazing has resumed (L'Huillier & Aislabie, 1988; Nie et al., 1999; Tozer et al., 2020), there has been little work done on understanding the below ground changes in the roots and how long the response lasts for. Existing work has shown changes in root biomass can be related to changes in above ground biomass, this has been used to predict root growth in several plant species, including agricultural grasses (Kuyah et al., 2012; Troughton, 1960). However, the accuracy of predicting root growth from above ground growth has been debated (Hu et al., 2018; Sileshi, 2014). Therefore, to understand how long lasting the effects of any increase in root biomass may be once standard grazing of grass pasture resumes, there is a need for physical data collection.

Roots are known to be important for plant survival. A larger or deeper root system potentially provides better anchorage against stock pulling (Thom et al., 2003) as well as improving uptake of water and nutrients needed for productive growth and supporting the soil microbiome (Badri & Vivanco, 2009; Gilroy & Jones, 2000). Other possible benefits of a larger root system includes influence on the mechanical properties of soil (Saleem et al., 2020; Uteau et al., 2013), additional organic matter inputs and increased carbon sequestration (Saleem et al., 2020), more efficient use of fertiliser inputs with reduced leaching (Moir et al., 2013) and increased tolerance to drought (Chloupek et al., 2010).

In established pasture where there is a mix of species interacting, there is no simple way of identifying the origin plant of each complex root system, or of measuring the root response of different species to changes in grazing scenarios. In addition, many differing variables and species interactions occur at multiple levels in a given field site which complicates the monitoring of ryegrass responses to deferred grazing alone (Nelson & Moser, 1994). In order to measure the short- and long-term response of perennial ryegrass to differing defoliation frequencies, a study under controlled conditions provides the means to better understand potential responses occurring in field site trials.

A glasshouse based study would therefore be beneficial by allowing for the control of variables that may influence ryegrass growth in pasture separate from grazing. Using simulated grazing by cutting grass to the same height, the impact

of delayed defoliation alone on ryegrass tillers and roots can be observed. Also, by growing a monoculture of ryegrass in pots, changes in root mass can more easily be observed with confidence that the roots analysed belong to the target species. Therefore, the glasshouse trial was proposed alongside a field-based trial to more accurately assess whether the responses seen in the field were a result of changes to grazing management.

The proposed study tested the hypothesis that root biomass will increase as above ground biomass increases when defoliation of ryegrass leaf is delayed, and that this increase in root biomass will be lost after standard defoliation resumes.

## **4.2 Materials and Methods**

### **4.2.1 Plant material**

The trial took place within the Ngahere glasshouse complex at the AgResearch Ruakura Centre in Hamilton. Sods of vernalized ryegrass were taken from a Lincoln pasture that had been previously sown with Samson AR37, two months prior to trial measurements commencing. The ryegrass tillers were then separated by hand into individual adult tillers with roots attached. The roots and leaf of these tillers were trimmed to approximately 40 mm with the aim of planting tillers of similar size. The individual tillers were planted in groups of two into eight trays which were prepped with sand and split into rows with dividers to allow for easy distinguishing between tillers. The sand trays were left in the glasshouse and hand watered for another 7 weeks before the tillers were transplanted into 1000 mm long root tubes.

A wooden framework was setup to hold the 1000 x 150 mm root tubes in the corner of a glasshouse. The root tubes were made from plastic piping that was cut in half and secured together with 48 mm cloth tape. To keep soil in the tubes in place, while still allowing for drainage, 1000 mm long plastic pot liners with drainage holes were used in each tube which were further supported by a floor made up of 25 mm of pea gravel. To prevent sand being lost from the tubes, a layer of 25 mm polyurethane foam was placed between the root tubes and pea gravel. The framework was covered in foil insulation to help stabilise the temperatures the tubes would be exposed to throughout the trial (Figure 4.1).

To fill the root tubes, sand and topsoil was sourced from Captain Compost, Frankton. The topsoil was a standard brown topsoil sourced from within 50 km of Hamilton and screened through 5 mm mesh. The sand was a washed river sand and was not tested. The topsoil was tested for standard nutrients and pH by Hill Laboratories as well as sulphate sulfur, organophosphates and asbestos which were all at safe levels or not detected. The standard topsoil test results showed pH and all tested for nutrients within the low range for Hill Laboratories assigned ranges with a pH of 5.7 and Olsen P of 14. To improve the soil for plant growth, an application of lime equivalent to 1000 kg/ha and 5 g Osmocote ® Exact were added to the soil surface of each tube before transplanting.

The tubes were filled in three layers with each layer being equivalent to 6 litres to mimic a soil profile, the layers were a sand bottom, a 50:50 sand and soil mixed middle layer, and a soil only top layer. The tubes were then watered every day for two weeks to allow for soil settling and weed germination before transplanting.

For transplanting, the ryegrass ramets were removed from the sand trays and trimmed to 10 cm, and any excess sand was washed off. Three ramets were transplanted at equidistant planting positions in each pot. Plants were watered twice a day for the first week and daily thereafter with tubes receiving approximately 50 mm at each watering.



Figure 4.1: Setup of root tubes showing the different defoliation treatments

#### 4.2.2 Experimental design

Four treatments were assigned across 204 total root tubes. All plants were defoliated to 10 cm at establishment. Timing of defoliation treatment was based on when defoliated plants reached the 2.5-3.0 leaf stage to simulate recommended rotational grazing practices. The applied treatments were assigned as:

- i). Def x0: no delayed defoliation. Ryegrass ramets were cut after ramets were established at every 2.5-3 leaf stage to simulate grazing.
- ii). Def x4: 4 weeks delayed defoliation. Ryegrass ramets were left to grow from establishment until December, at which point plants were trimmed and maintained at the 2.5-3 leaf stage under simulated grazing.
- iii). Def x8: 8 weeks delayed defoliation. Ryegrass ramets were left to grow until January, at which point plants were trimmed and maintained at the 2.5-3 leaf stage under stimulated grazing.
- iiii). Def x12: 12 weeks delayed defoliation. Ryegrass ramets were left to grow until February, at which point plants were trimmed and maintained at the 2.5-3 leaf stage under stimulated grazing.

The trial was designed with 6 reps and 7 cohorts arranged in a randomized complete block design. At each defoliation interval, a cohort of 24 root tubes were destructively harvested to obtain a timeline of changes in plant biomass over the reproductive and recovery period. Continuous measurements were applied to the remaining pots.

#### 4.2.3 Glasshouse measurements

To measure glasshouse conditions, PAR readings and temperature were recorded. PAR readings were taken with a sensor (LI-250A Light Meter, LI-COR) placed in the centre of each root tube at the soil surface and facing up. PAR readings were taken just after noon on a cloudless day when possible after each delayed defoliation interval had ended. Care was taken not to disturb the leafy material and the sensor reading was averaged over a 15 second period. Four dataloggers (DS1922L iButton, Maxim Integrated) were installed to record temperature every hour throughout the course of the trial. Each temperature datalogger was placed in

the centre of a root tube at the soil surface level and in a different treatment for each block. At each period where a cohort was destructively harvested, the temperature dataloggers were moved to the next cohort and the same treatment that they were in previously.

The root tubes were kept well-watered and the amount of water given to each root tube was not altered between treatments. Root tubes were watered once a day with a sprinkler attachment at the end of the hose, this was held over each root tube for a few seconds. Water given was estimated using manual rain gauges which were installed within each block and caught on average 50 ml at each watering.

#### 4.2.4 Harvest measurements

The timing of destructive measurements and defoliation treatments were determined based on when defoliated plants reach the 2.5-3.0 leaf stage, to replicate recommended grazing strategies (Table 4.1). All plants were cut to 10 cm in October during trial establishment. The trial began in November when Def x0 plants were at the 2.5-3.0 leaf stage. This is when Def x0 plants were first defoliated and the first destructive harvest was completed to measure leaf, stubble and root biomass. Non-destructive measurements were made at the same time. This included the counting of live, dead and reproductive tillers as well as the gathering of leaf material removed by defoliation for the Def x0 plants. Leafy material was not removed from any other treatment plants at this stage except for those in the destructive cohort.

In December, when Def x0 had reached the 2.5-3.0 leaf, the next set of measurements was conducted. At the same time, Def x4 was defoliated. This procedure was continued into January when Def x0 and Def x4 plants had reached the 2.5-3 leaf stage. At this time, Def x8 plants were defoliated and all other measurements were repeated. In February when Def x0, Def x4 and Def x8 were at the 2.5-3 leaf stage, Def x 12 was defoliated and all other measurements repeated. After February, all plants had been defoliated and continued to be maintained under routine simulated grazing, with measurements continued at each 2.5-3.0 leaf stage until September. This is with the exception of the space between measurements taken in February until measurements in late April due to the Covid-19 lockdown, leaf stage averaged 3.2 leaves per plant at the April harvest.

Table 4.1: Timing of destructive measurements as well as the delayed defoliation interval for each treatment. H1-H7 refers to when a destructive harvest was done on cohort, as well as continued measurements on the remaining pots. The closed period (dark grey) is the time each treatment was locked up for and the following response period (light grey) is the period after treatment application for which ongoing measurements continued. The black X denotes the month in which defoliation for each treatment was applied. No measurements happened from February to late April/early May due to Covid-19 lockdown. No measurements happened in June or August because plants had not yet reached the 2.5-3.0 leaf stage.

	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20	Apr-20	May-20	Jun-20	Jul-20	Aug-20	Sep-20
Ramets transplanted		H1	H2	H3	H4	-	-	H5	-	H6	-	H7
Treatments	Closed		Response									
Def x0		X										
Def x4			X									
Def x8				X								
Def x12					X							

Before November measurements, endophyte blots were taken for one tiller per plant. Endophyte infection frequency was determined using the immunoblot assay described by Hahn et al., (2003). Each tiller was cut with a sharp scalpel and the base firmly pressed against blotting paper. The blots were stored in a sealed envelope at 4 °C and processed within a week.

After the December harvest, shade cloth collars were placed around the remaining undefoliated plants for treatments Def x8 and Def x12. The collars were designed to prevent the longer grass from falling over and shading neighbouring pots were grazing simulation had been applied. The collars also shade the deferred tillers so that they were not exposed to more light than would be found in a pasture situation. The strength of the shade cloth used was decided in a pre-trial test by taking multiple PAR sensor readings in a pasture and in the glasshouse to find the most representative level of shading for tillers in deferred pasture.

On each measurement date the number of tillers was counted for one plant from each root tube, and the reproductive stage of each tiller recorded. The plant was marked with an adjacent tag and tillers of the same plant were counted throughout



the trial. Dead tillers were counted separately at the same time as live tillers. The criteria for a dead tiller was no growth after being defoliated, or wilting with no green tissue apparent anywhere on the stem. The reproductive stage of each tiller was assigned to one of five categories. R0: a node present but no seed head. R1: node present and an enclosed seed head. R2: node present and a partially emerged seed head. R3: seed head fully emerged, but flowers had not yet opened. R4: fully emerged seed head with open or dropping flowers. These categories were based on the reproductive stages of flowering as described by Moore et al. (Moore et al., 1991).

After the non-destructive measurements, the root tubes that required defoliation treatment were cut and the removed biomass was collected. The three plants within each root tube were all cut to the same height. Defoliation treatments were applied using a 50 mm diameter plastic ring placed at the soil surface to provide a consistent height for cutting and to replicate the grazing height of cattle. This occurred for all plants within root tubes where simulated grazing was being applied and all plants within the cohort to be destructively harvested on that date. After defoliation the cut material was oven-dried at 65°C for 24 h and weighed.

For the 24 root tubes used for the destructive cohort measurements, the stubble was also collected after the leafy material was cut. The stubble was cut level with the soil surface with a scalpel blade and collected. The stubble was oven-dried at 65°C for 24 h and weighed.

After the stubble harvest the root tubes were opened and the roots divided into three different masses per tube according to their soil layer. The roots were extracted by removing the bulk of the soil using a 50 mm sieve for the top and middle layer and a 20 mm sieve for the sand layer followed by more careful washing with a 2 mm sieve to prevent root loss, then oven dried at 65°C and weighed.

During the processing of the root tubes, soil was sampled from each layer to obtain gravimetric soil moisture content throughout the root tube profile. A small amount was taken from the top, middle and bottom of a soil layer to obtain a representative sample. A fresh weight for each soil sample was recorded before

the samples were dried at 100°C for 48 hrs. The soil was reweighed and the difference in weights was used to calculate soil moisture.

#### 4.2.5 Statistical analysis

This trial used a split plot design and was analysed using Genstat 20th edition VSN International for an analysis of variance (ANOVA). Data was analysed for interactions across harvest and by treatment for non-destructive measurements and then for interactions within each harvest for both non-destructive and destructive measurements. Data presented is for treatment effect within each harvest.

### 4.3 Results

#### 4.3.1 Glasshouse conditions

Plants in the glasshouse were exposed to warmer ambient temperatures than plants outside over the same time period. The glasshouse temperature did not decrease to freezing during the winter months, with the lowest recorded glasshouse temperature being 3.6°C in July (Table 4.2). The glasshouse also experienced high temperatures in the summer months. The average temperatures ranged between 20-22 °C during summer, but maximum temperatures were much higher, with the maximum recorded temperature being 46.1 °C in December. Temperature maximums above 40 °C were also recorded in January and February.

There were no differences in soil moisture content between delayed defoliation treatments (Figure 4.2). In December the delayed defoliation treatments (Def x4, Def x8, Def x12) had a decreased soil moisture content compared to the control treatment (Def x0) ( $P=0.015$ ) suggesting a greater water use by the deferred plants at this time.

Prior to the first defoliation treatment, all treatments were receiving similar PAR levels (Figure 4.3). Shade cloth covered collars were applied in December. Afterwards PAR decreased in delayed defoliation treatments Def x8 and Def x12 compared with defoliated treatments Def x0 and Def x4 ( $P<0.001$ ).

Table 4.2: Glasshouse temperature taken at soil surface level between November 2019 – July 2020.

Date	Average Temperature (°C)	Minimum Temperature (°C)	Maximum Temperature (°C)
Nov-19	20.99	11.61	34.62
Dec-19	22.38	13.11	46.08
Jan-20	23.18	13.61	43.09
Feb-20	24.2	14.62	41.1
Mar-20	21	10.1	37.61
Apr-20	17.37	8.59	34.62
May-20	15.62	5.58	27.63
Jun-20	14.21	7.59	23.63
Jul-20	12.43	3.57	20.63

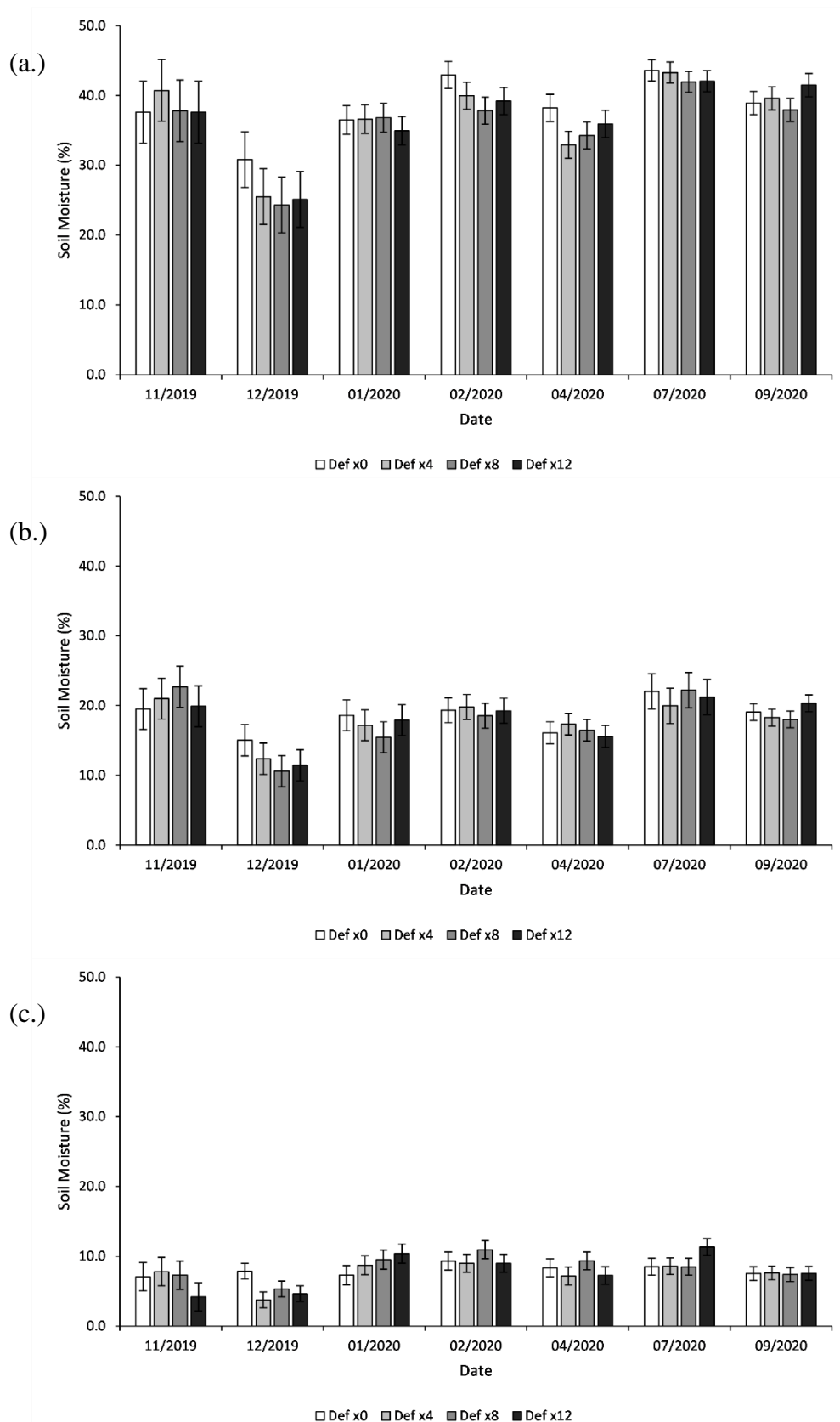


Figure 4.2: Gravimetric soil moisture content (%) of soil in root tubes throughout the trial. (a). Soil moisture for the top layer, a 35cm deep brown top soil. (b). Soil moisture percentage in the second 35 cm soil layer. A 50:50 mix of brown topsoil and river sand. (c). Soil moisture percentage in the bottom 35 cm layer of river sand. Standard error bars are shown (ANOVA)

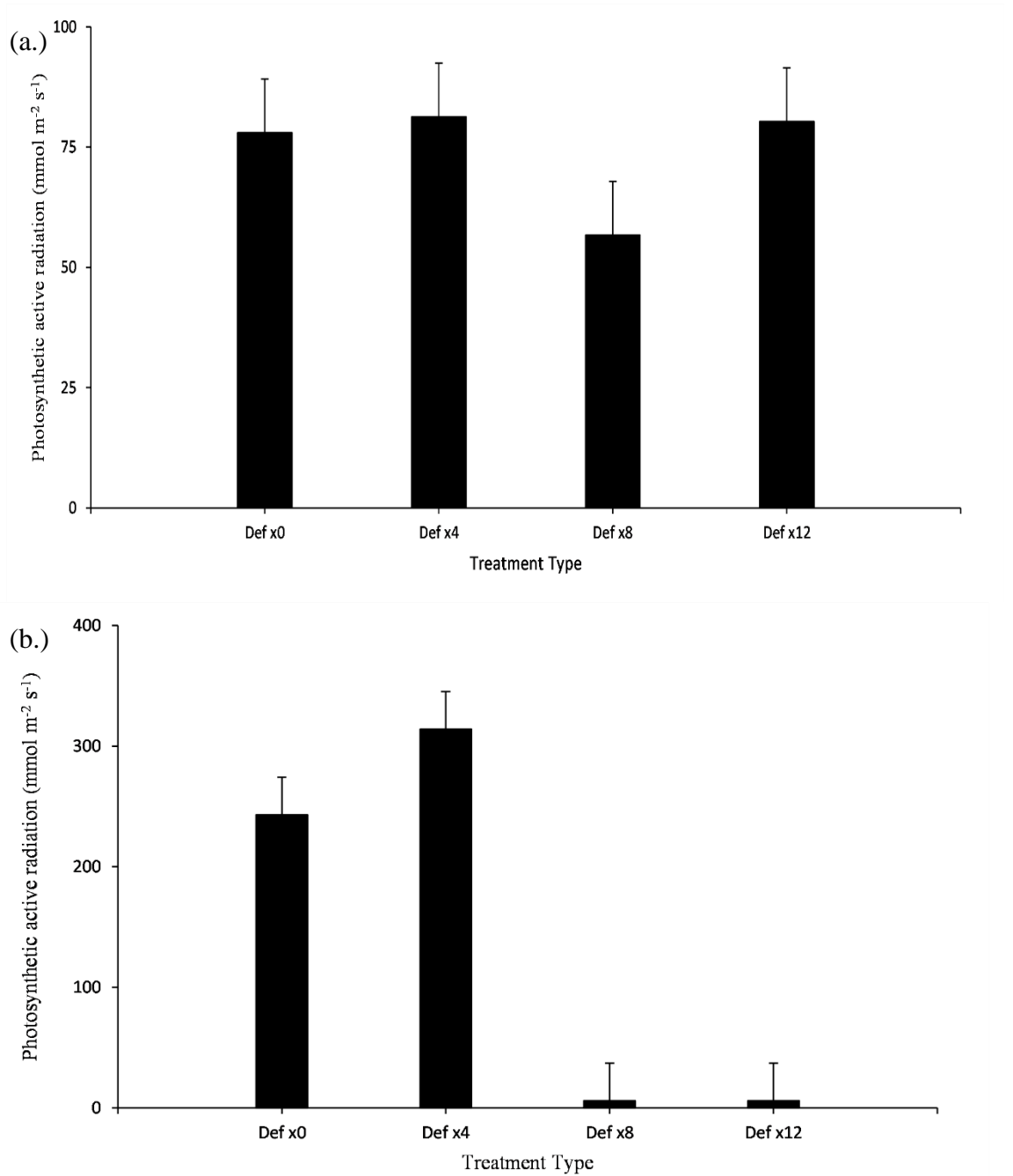


Figure 4.3: Photosynthetically active radiation (PAR) at the soil surface within each root tube. (a) PAR in November prior to Def x0 defoliation when all plants were at the 2.5-3 leaf stage and no shading had been added. (b.) PAR readings in December after the defoliated (Def x0) and four week delayed defoliation (Def x4) treatments had been cut, and shade cloth collars applied to eight (Def x8) and 12 week (Def x12) delayed defoliation treatments. Standard error bars are shown (ANOVA).

#### 4.3.2 Tiller Production

The number of live tillers per plant did not differ between treatments in November (Figure 4.4). In December there were more tillers in the delayed defoliation treatments compared to the control ( $P = 0.013$ ). In February the number of live tillers was again similar across all treatments, averaging 26 tillers per plant, and there were no further differences between any of the treatments in late April, July or September.

Treatments with a longer delayed defoliation interval decreased in percentage of live tillers over time ( $P < 0.05$ ; Figure 4.5). There were no differences in the percentage of live tillers between treatments in November, December or January when the mean percentage of live tillers was higher than 90% for all treatments ( $P > 0.05$ ). After January, the percentage of live tillers for Def x0 was either the same or higher than the other treatments. Def x12 had the largest decrease in percentage of live tillers and was significantly lower than other treatments from late April to September ( $P < 0.05$ ).

A delayed defoliation interval allowed for a higher number of reproductive tillers per plant (Figure 4.6). Reproductive development began in November with node formation present in all treatments. Reproductive tillers with seed heads were present between December and the end of February. As the defoliation interval increased, the number of reproductive tillers increased. The Def x12 treatment had the highest total of reproductive tillers in February ( $P < 0.001$ ). There were more reproductive tillers in Def x8 and Def x12 than in Def x0 and Def x4 in December and January ( $P < 0.01$ ). Reproductive tiller growth stopped for Def x8 treatment after January and all treatments after February.

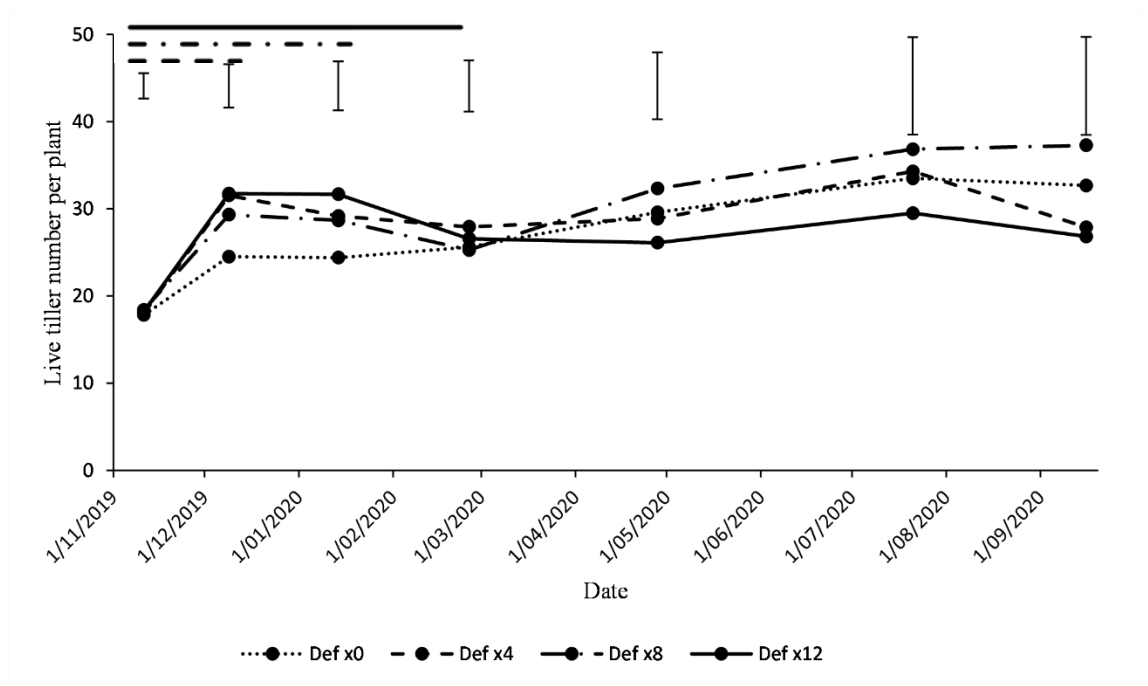


Figure 4.4: Effect of a delayed defoliation interval on the number of live perennial ryegrass tillers per plant. Plants were defoliated at the 2.5-3.0 leaf stage (Def x0), or after a four (Def x4), eight (Def x8) or 12 (Def x12) week defoliation interval. After this period, all treatments were defoliated when 2.5-3.0 new leaves were produced. Horizontal bars represent the extended defoliation intervals for the Def x4, Def x8 and Def x 12 treatments. Standard error bars are shown (ANOVA).

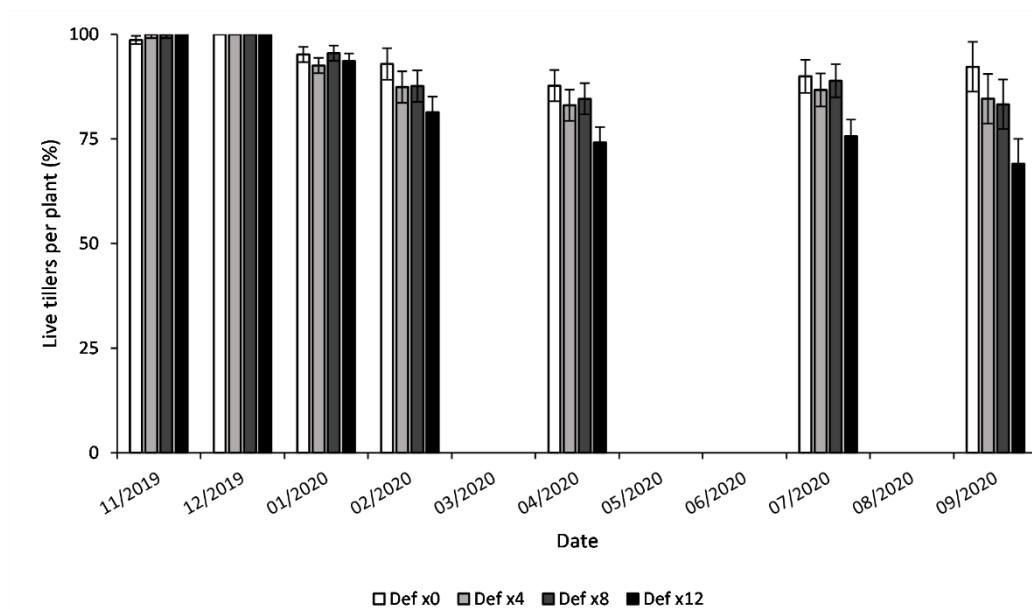


Figure 4.5: Effect of a delayed defoliation interval on the percentage of live perennial ryegrass tillers over time. Plants were defoliated at the 2.5-3.0 leaf stage (Def x0), or after a four (Def x4), eight (Def x8) or 12 (Def x12) week defoliation interval. After this period, all treatments were defoliated when 2.5-3.0 new leaves were produced. Standard error bars are shown (ANOVA).

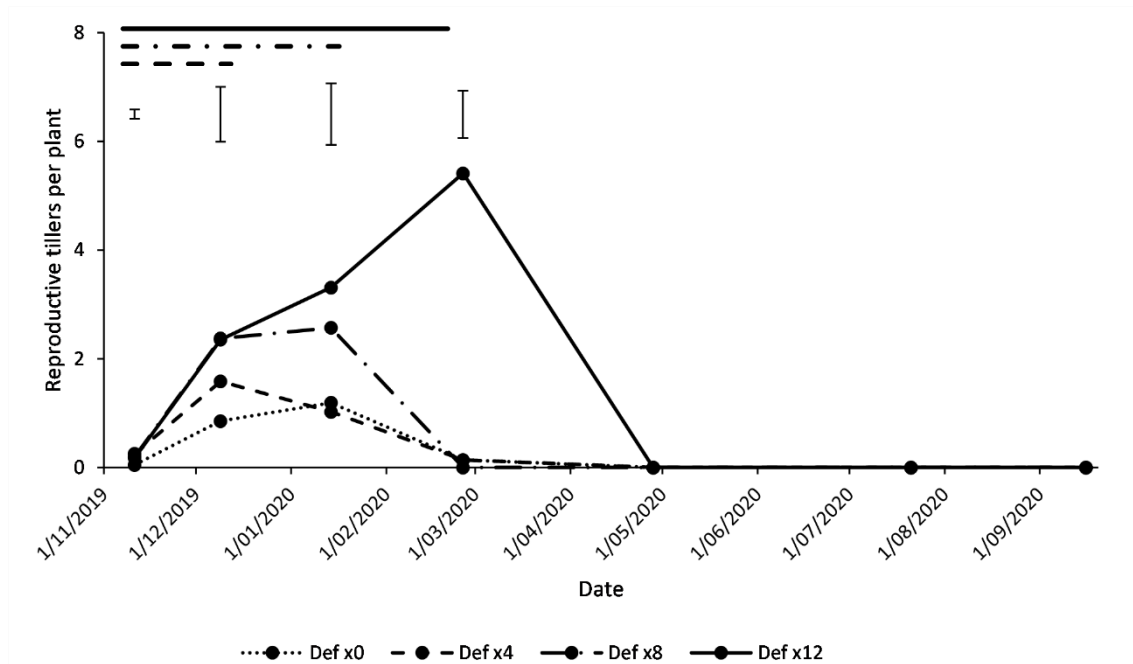


Figure 4.6: Effect of an extended defoliation interval on the number of reproductive perennial ryegrass tillers per plant. Plants were defoliated at the 2.5-3.0 leaf stage (Def x0), or after a four (Def x4), eight (Def x8) or 12 (Def x12) week defoliation interval. After this period, all treatments were defoliated when 2.5-3.0 new leaves were produced. Horizontal bars represent the extended defoliation intervals for the Def x4, Def x8 and Def x 12 treatments. Standard error bars are shown (ANOVA).

#### 4.3.3 Plant above-ground biomass

All treatments where defoliation was delayed had their highest peak in leaf dry weight above 5 cm prior to the end of the delayed defoliation interval (Figure 4.7). The control treatment Def x0 had a peak in leaf biomass during December and then produced a relatively constant leaf biomass throughout the following months with similar weights in January, May and July ( $\pm 0.1$ ). Leaf biomass was greater in delayed defoliation treatments than the control in December ( $P < 0.001$ ). After delayed defoliation intervals ended between December to February, treatments had similar biomass to the control. In May, Def x4 had the lowest harvested leaf biomass compared to all the other treatments ( $P = 0.045$ ). By the end of the trial, leaf biomass tended to be lower in treatments with longer delayed defoliation intervals and was significantly lower for Def x12 in September ( $P = 0.039$ ).



There were no differences found in stubble weight throughout most of the trial except for September measurements. In that month, stubble dry weight was lower in Def x12 than all other treatments with 2.8 g vs an average of 4.1 g for Def x0, Def x4 and Def x8 ( $P=0.029$ ).

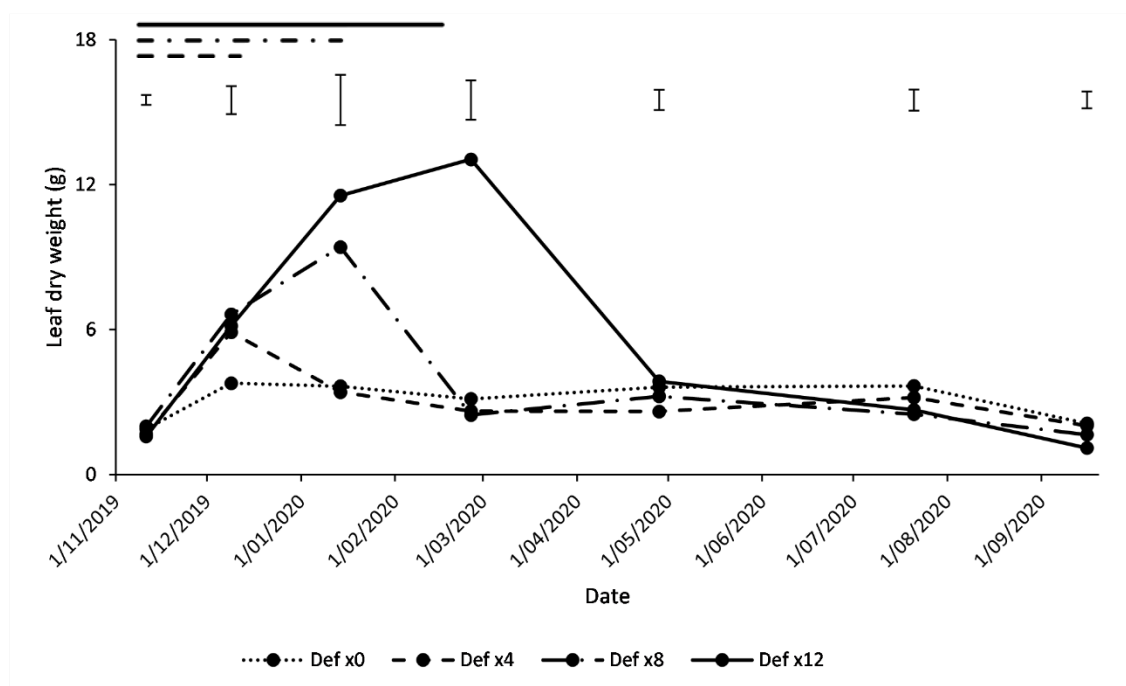


Figure 4.7: Effect of an extended defoliation interval on the leaf biomass above a 5 cm trimming height. Plants were defoliated at the 2.5-3.0 leaf stage (Def x0), or after a four (Def x4), eight (Def x8) or 12 (Def x12) week defoliation interval. After this period, all treatments were defoliated when 2.5-3.0 new leaves were produced. Horizontal bars represent the extended defoliation intervals for the Def x4, Def x8 and Def x12 treatments. Standard error bars are shown (ANOVA).

#### 4.3.4 Root biomass

Treatments with a delayed defoliation interval had between 70 and 100% higher average total root biomass in December and January compared to the control treatment, but variability in root biomass was high and these differences were not significant (Figure 4.8;  $P>0.05$ ). After January total root biomass declined in all treatments to similar levels.

The root biomass within each of the three soil layers followed a similar trend to the total root biomass. There were no significant differences between treatments for the top soil layer or the second soil and sand mix layer ( $P>0.05$ ). The control

treatments had a root biomass for the bottom sand layer in late April which was between 70 to 80% lower than the other treatments, while Def x4 and Def x12 had the highest means for lower root biomass (Figure 4.9;  $P < 0.05$ ).

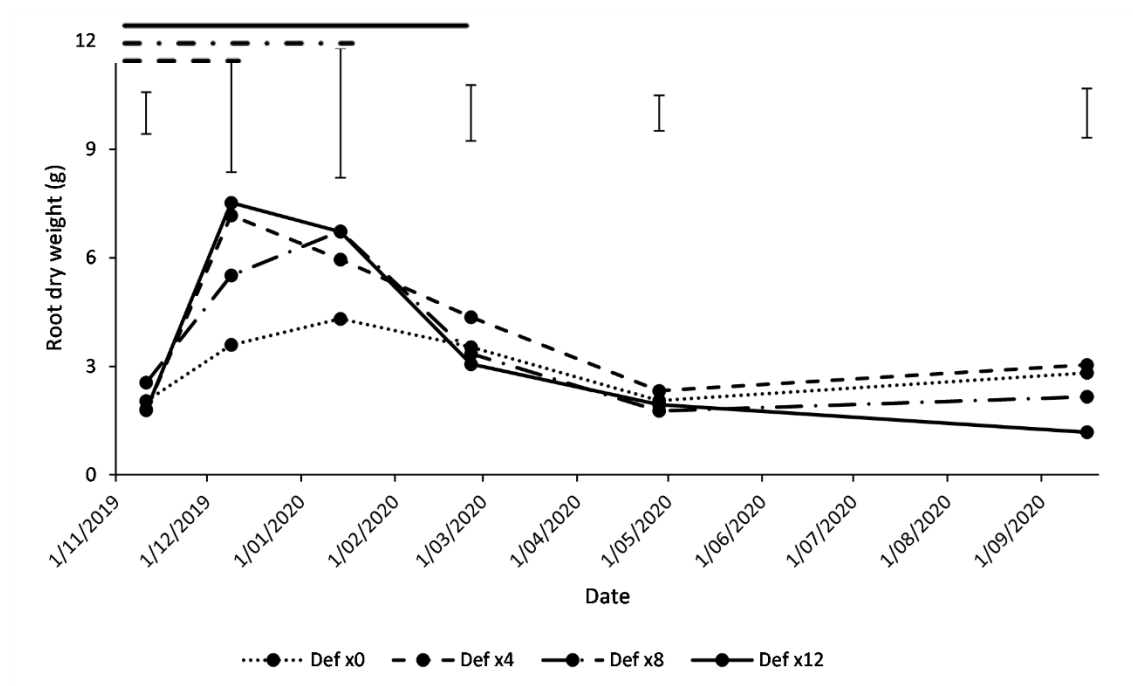


Figure 4.8: Effect of an extended defoliation interval on total dry weight of perennial ryegrass roots extracted from root tubes with 3 plants per tube. Plants were defoliated at the 2.5-3.0 leaf stage (Def x0), or after a four (Def x4), eight (Def x8) or 12 (Def x12) week defoliation interval. After this period, all treatments were defoliated when 2.5-3.0 new leaves were produced. Horizontal bars represent the extended defoliation intervals for the Def x4, Def x8 and Def x12 treatments. Standard error bars are shown (ANOVA).

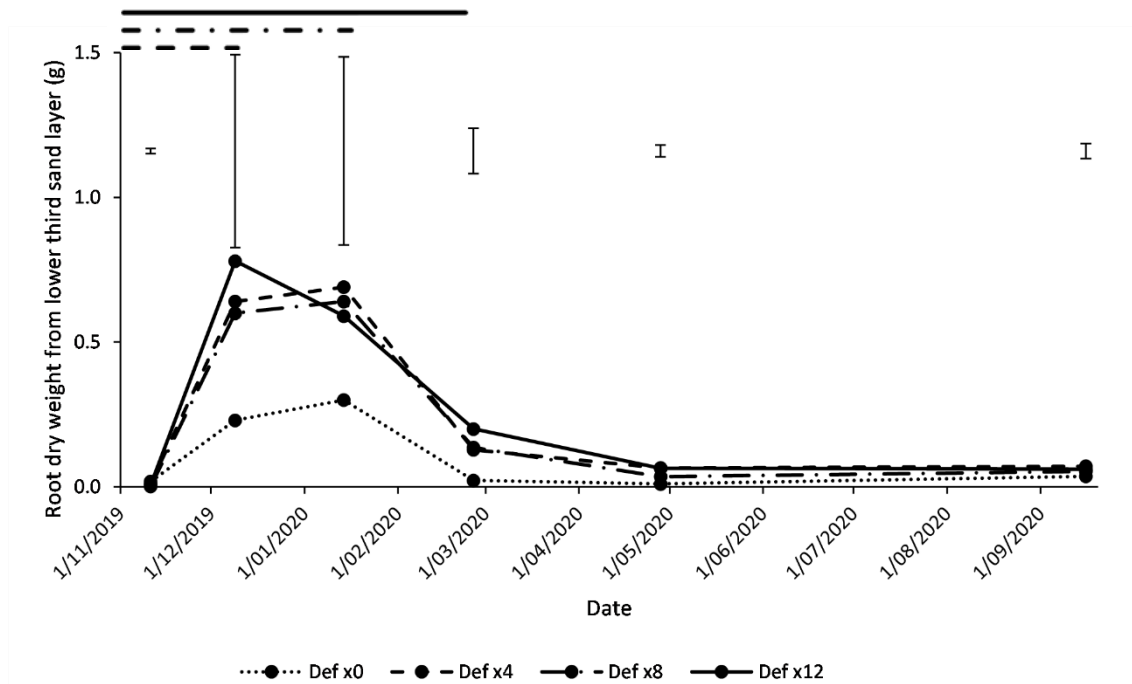


Figure 4.9: Effect of an extended defoliation interval on biomass of perennial ryegrass roots extracted from the bottom third sand layer of root tubes with 3 plants per tube. Plants were defoliated at the 2.5-3.0 leaf stage (Def x0), or after a four (Def x4), eight (Def x8) or 12 (Def x12) week defoliation interval. After this period, all treatments were defoliated when 2.5-3.0 new leaves were produced. Horizontal bars represent the extended defoliation intervals for the Def x4, Def x8 and Def x 12 treatments. Standard error bars are shown (ANOVA).

#### 4.4 Discussion

The results found by this study were that perennial ryegrass plants under the conditions they were subject to during this glasshouse trial received no long-term benefit from any period of delayed defoliation. The treatments where defoliation was delayed initially produced a higher biomass in both leafy material and greater tiller numbers with more developing seed heads. Once defoliation resumed, treatments with longer delays in defoliation had higher tiller mortality and lower leaf biomass with no significant change in number of alive tillers. There was a possible increase in root biomass, particularly in lower soil layers but the effects were not significant for this experiment. Overall, this suggests that a period of delayed defoliation in perennial ryegrass can produce an increase in above and below-ground biomass, tiller number and reproductive development but these benefits did not persist in the glasshouse setting once defoliation was resumed.

Other glasshouse based studies used standard defoliation rates for grazing practices and found ryegrass to be sensitive to defoliation below the 3 leaf stage resulting in decreases in dry matter yield or regrowth (Fulkerson et al., 1994). Previous research compared a four and eight week defoliation interval across a range of different grass species. The four week defoliation interval which most closely compares to the control (Def x0) for this study had half the dry matter yield and tillers of the longer interval (Hill & Pearson, 1985). These results are consistent with the first part of this study (November-February) although the differences found by Hill and Pearson (1985) were larger. Part of the reason why the control for this study performed better than plants under similar defoliation rates in other studies may be because the timing of defoliation was based on growth rate and not a set number of days, allowing for more recovery in the control treatment. There was also an unplanned delay in defoliation for all treatments from February to late April, with the April defoliation occurring approximately 2-3 weeks later than expected based on the 2.5-3.0 leaf stage. After this time, the control performed either slightly better or the same as other treatments. The results of this study where tillers in delayed defoliation treatments had higher mortality and lower biomass than the control also differs from previous field studies (McCallum et al., 1991; Nie et al., 2005) where tiller density of ryegrass increased following defoliation after a period of deferment. Long term studies in Australia (Nie et al., 2014) have also found longer lasting increases in dry matter production after a deferment period than was suggested by the results of this glasshouse study.

In studies where root mass of perennial ryegrass has been investigated, a decrease in total below-ground biomass has been closely linked to defoliation frequency and intensity (Ennik & Hofman, 1983; Evans, 1973). Another study observed a decrease in root mass in a different perennial grass species that occurred less than a week after defoliation (Gomide et al., 2019).

Deferred treatments in this study did initially have higher root masses than the non-deferred treatment. This has also been observed in other literature where comparison has been made between pasture left to rest and continuously grazed. (Chen et al., 2015; Oates et al., 2011). Peak root mass happened in deferred plants between December and January and fell in February, the decline in

February happened in all treatments including the yet to be cut 12 week deferred treatment (Def x12) which suggests the decrease here was not related to timing of defoliation. Root biomass was lowest in late April but increased again by the end of the trial in September. This increase may be caused by the seasonal patterns of root growth. Previous studies have identified a peak in ryegrass root growth in August or early spring (Matthew, 1996).

Patterns in seasonal growth alone does not explain the decline in biomass between February and late April, as this is when an autumn peak in root growth would otherwise be expected (Wedderburn et al., 2010). Colder weather and less light in winter can restrict root growth, but glasshouse temperatures never fell lower than 3°C in the coldest months. The causes of this trend are uncertain as the glasshouse was not accessible during this period. Possible factors that could contribute to restricted root growth in plants include the mechanical properties of the soil, high or low moisture content, pH, temperature and nutrient availability (Dracup et al., 1992; Huang et al., 1998; Unger & Kaspar, 1994; Yan et al., 2013). Some yellowing of the leaves was noted in late April/May, suggesting that the plants had been impacted by nutrient deficiency while unattended. It is also possible that the loss in roots is related to heat stress either as a delayed response to late summer temperatures that reached 41 °C and averaged 24°C in February. March was also warm with plants exposed to up to 38°C and an average of 21°C. Growth rates of ryegrass have been found to decrease at temperatures above 20°C (Hunt & Field, 1978), this means glasshouse conditions were hot enough to restrict the potential growth of the plants in this study. In this trial, tiller number, root biomass for all treatments and top biomass for all but Def x12 decreased from previous months in February. Heat stress may have cause restricted growth over this period as the temperature in the glasshouse reached levels outside of the optimum range for ryegrass regrowth (Hill & Pearson, 1985). It is possible heat stress acted as a limiting factor which lessened the differences seen between treatments. Additional effects of high temperature resulting in limited nutrient uptake are also possible. Other work has described a similar result in other grass species where root mass decreased after flowering and exposure to high soil temperatures that in turn affected nutrient uptake (Gavito et al., 2001). This is relevant to the glasshouse study as the root tubes were positioned above-ground

and while they were shielded to avoid exposure to light, they could not be buried in a true replication of below-ground temperatures.

Growth rate in leaf biomass slowed from February onwards. This was potentially caused by a combination of seasonal reductions in growth as well as heat stress. Growth rate in ryegrass has been found to decrease at temperatures up to 25°C following defoliation (Slack et al., 2000). There was no compensatory increase in growth observed in this study after defoliation despite it being observed in other studies where grazing or other stress treatments have been combined (Gastal et al., 2010; Tozer et al., 2017). Biomass was also similar across treatments once defoliation had occurred.

It is also notable that leaf biomass accumulated between defoliation intervals was lower in early spring, while root biomass was increasing. This suggests that in early spring, more growth was allocated to roots rather than leaves. This is supported by other studies that found that spring is a key period for root growth for perennial ryegrass (Dodd & Mackay, 2011). Water soluble carbohydrate reserves have been linked to the size of the root system (Donaghy & Fulkerson, 2002). Perennial ryegrass stores part of the water soluble carbohydrate reserves near the stubble, which in this experiment was maintained to 5cm and could have supported the observed root growth (Lee et al., 2009). However, growth of photosynthetic tissue is typically favoured over root growth. Other studies have reported that root growth is stopped after grazing until the plant reached the 2 leaves per tiller stage of growth (Fulkerson & Donaghy, 2001). The experimental plants would have reached this stage, as harvesting of leaf material occurred only at the 2.5-3 leaves per tiller stage. In addition, live tiller numbers were either higher or unchanged from previous harvesting despite lower biomass in later harvests. This suggests a possible decrease in tiller weights coinciding with a period of greater root growth.

The plants with a 12 week delayed defoliation interval began to differ from other treatments later in the trial with lower root weight, lower leaf and stubble biomass and a higher percentage of dead tillers, these differences were most obvious in September when the trial ended. However, the total number of live tillers for Def x12 remained similar to the other treatments. Possible reasons for this difference

could be that newer and therefore smaller tillers were replacing old tillers which had died. The death of reproductive tillers could also be a factor for the higher percentage of dead tillers found in Def x12 and may explain the lack of difference in total live tiller number. Studies have found tiller appearance does begin as early as September, which is prior to the tillers gaining the weight of reproductive stems (Hernández-Garay et al., 1993). Only mature adult tillers were counted in this trial but attached immature daughter tillers were observed. Def x12 produced the highest number of reproductive tillers. Other research found over half of reproductive tillers produced daughter tillers and that reproductive tillers usually died once defoliation was applied (Laidlaw, 2005; Waller et al., 1999). This would explain the higher rate of mortality seen in the Def x12 treatment which produced the highest number of reproductive tillers per plant but did not have a significantly lower number of total alive tillers when compared to all other treatments.

The Samson cultivar used for this trial is a mid-heading date (+3) cultivar with seed head emergence expected in late October. While appearance of reproductive tillers did begin in November with node formation, the emergence of seed heads did not occur until later. The main reproductive period in this trial occurred between January and February where the number of reproductive tillers was highest, and the majority of the seed heads had reached maturation. This differs from other studies where the reproductive period of ryegrass is concentrated in late spring/early summer (Hernández-Garay et al., 1997; Hunt & Field, 1978). Later emergence of seed heads into winter does occur when earlier production is restricted by grazing or climate (Korte, 1986; Waller et al., 1999). In this trial, the strict trimming of the ramets pre-trial could have restricted earlier reproductive growth. Allowing for the full maturation of the seed head that happens in the delayed defoliation treatments creates an opportunity for seedling recruitment that is prevented under frequent defoliation (L'Huillier & Aislabie, 1988). It is expected that the ideal timing for a deferment period would vary when cultivar and climate conditions affect the timing of reproductive development.

Initially, leaf biomass, root biomass and tiller numbers were all higher in treatments where defoliation was delayed, but these differences did not last once the delayed defoliation interval ended. Under the conditions these plants were

exposed to there was no long-term benefit of delayed defoliation on perennial ryegrass. The results of this trial further support the hypothesis that while root biomass does increase when defoliation is delayed, the increase may not be sustained under standard defoliation practices.

Further research is recommended to test whether there are any benefits to increased root biomass for a limited time that extend beyond the period of deferment such as the influence on the soil biome or increases in soil organic matter from root turnover. It is unknown if the differences seen in Def x12 at the end of the trial would have continued to with further defoliation intervals. It is also likely that by allowing the full maturation of reproductive tillers, there will be an increase in the seed bank. This effect was not considered in this study but could cause beneficial changes in field pasture conditions after grazing has resumed. Research into the long term effects of having reproductive tillers mature could be achieved with a longer running study.

#### **4.5 Conclusion**

Root biomass, leaf biomass, total live tiller number and reproductive tiller number can increase under a period of delayed defoliation over the reproductive period in perennial ryegrass. Root biomass was highly variable between plants but a temporary increase in root biomass at depth did occur in treatments with delayed defoliation. While longer periods of delayed defoliation had increasing leaf biomass under delayed defoliation, the regularly defoliated treatment performed the same or better in leaf biomass once standard defoliation had resumed for all treatments. Plants with a longer period of delayed defoliation (12 weeks) had a higher percentage of dead tillers and the lowest leaf biomass by the end of the trial though number of alive tillers was the same between treatments. There were no other significant differences across treatments once defoliation resumed. Possible reasons given for the loss of differences between the control and treatments subjected to a delayed defoliation interval after standard defoliation resumed was the longer defoliation interval between February and late April, or climatic variables including heat stress in February affected later growth of plants. The results of this study suggest that the potential benefits of delaying defoliation for perennial ryegrass in biomass or tillers may be lost once standard defoliation



resumes or when plant growth is limited by other variables. Further research under controlled conditions on the effects of delayed defoliation is recommended, but with better control of above and below ground temperatures.

## **5 General Discussion**

### **5.1 Introduction**

Methods for adjusting grazing management strategies to improve pasture persistence for livestock are valuable to farmers trying to increase productivity of farms under the pressure of a changing climate. Improving the persistence of ryegrass based pasture by withholding grazing over the reproductive period has promise for increasing dry matter yields and tiller densities compared to standard rotational grazing (McCallum et al., 1991; Nie et al., 2005; Nie et al., 2014). The potential increase in the associated belowground biomass of pastures was speculated to be beneficial for improving survival of plants through enhancements in the efficiency of nutrient and water uptake (Haling et al., 2016; Peek et al., 2005). However, there is limited research on how the belowground biomass of ryegrass pasture is influenced by a period of deferment.

The main objectives of this research were to compare changes in above and below ground biomass and tiller production of perennial ryegrass in a controlled monoculture and in an established pasture scenario when different lengths of deferment were applied that aligned with the beginnings of the reproductive period (October). For the field trial the hypothesis tested was that aboveground dry matter and belowground biomass increases under pasture when deferred in spring and that this increase will be greater under an extended period of deferment ending in autumn. In the glasshouse trial, it was hypothesised that root biomass will increase as above ground biomass increases when defoliation of ryegrass leaf is delayed, and that this increase in root biomass will be lost after standard defoliation resumes.

Two trials were established to run alongside each other. One was a field based trial where changes in above and below ground biomass, botanical composition, ground cover, tiller densities and nutritive value was measured for three different treatments. Plots were either grazed under standard rotational grazing practices (control treatment), deferred from grazing from late spring till the end of summer, or deferred from late spring till the end of autumn, after which time standard grazing resumed. The second trial was glasshouse based and measured above and belowground biomass and tiller numbers of ryegrass monocultures. Root tubes

were established with four treatments. Simulated grazing by cutting of leafy material was applied to mimic standard rotational grazing, or pots were withheld from cutting for four, eight or twelve weeks. Both trials used an experimental split block design. The implications of the results are considered here.

## **5.2 Below ground biomass**

The aim for this study was centred towards investigating how plant roots were affected by different lengths of deferments from grazing. To obtain data, the roots were physically extracted from soil, either from the set limits of the root tubes or from root cores for pasture. Data obtained from the root tubes showed a trend for the deferred treatments to have greater total root biomass than the control treatment between December-January and a greater biomass at depth in late April. However, total root biomass extracted from both the root tubes or the soil cores was not significantly different between treatments throughout either trial. Trends over time for root biomass extracted from the root tubes also did not reflect what was found from the soil cores. The results from both trials contradict the findings of previous research from an unpublished root-tube based study where using the same extraction methods for obtaining root biomass found significant differences between treatments under different withholding periods. Soil core based root biomass also found no change between measurements while previous studies found variation in root biomass over time related to root turnover and seasonal changes (Saggar & Hedley, 2001).

Consideration must be given to why no difference was found in root biomass with or without a deferment period. For the soil cores, it is most likely that difficulties that arose in extracting the roots limited the ability to obtain enough data to overcome natural variation in an established pasture. Firstly, the data set was restricted because cores could not be extracted during the drought and pandemic resulting in a lack of data between December and May, over which time one deferred treatment returned to standard grazing. This means there is no data for the initial deferment period which coincides with the same period of time where potential differences were seen in the root tubes. Secondly, due to difficulties with the clay based soil, sodium hexametaphosphate 5% solution had to be used as a dispersal agent on the soil to extract roots. While care was taken that the cores

were exposed to the solution for a similar amount of time, it is uncertain how the solution or soaking period may have affected the fragility of the roots and therefore the ability for them to be extracted by sieving. Thirdly, it is possible that the coring method used was not sufficient to detect smaller differences due to the limits in depth and frequency at which they were taken. Added difficulty comes from the irregularity by which roots are distributed in the field between different species (Ozanne et al., 1965) when attempting to obtain a representative dataset.

However, because roots extracted from the root tubes also did not differ significantly when methods of root extraction successfully used in previous studies were applied (Crush et al., 2005), it is possible that any changes in root biomass under deferment were not significant. Other possible reasons for the lack of response include the effect of variables such as heat or nutrient stress. Both the glasshouse and the field site were exposed to temperatures high enough to restrict growth of perennial ryegrass over the summer period (Hunt & Field, 1978).

Nutrient stress or a combination of the heat and nutrient stress could also be a factor, as yellowing of leaves was noticed for both trials in May. Since roots are an important part of nutrient uptake, if growth of root biomass was restricted in summer, this could explain nutrient stress occurring later (Gavito et al., 2001).

Drought stress was also likely to be contributing factor to the lack of response in the field study. Considering these potential factors and results from previous research, the results of this study indicate that a period of deferment will not alter root biomass compared to standard rotational grazing when plant growth is restricted by other variables such as heat stress.

### **5.3 Above ground biomass**

While differences measured in belowground biomass were limited, effects of the deferment period were observed in aboveground biomass for both trials.

Accumulated leafy dry matter increased with deferment length, with the peak in standing above ground biomass occurring in late February in the glasshouse. In the field based study, the treatment withheld from grazing until autumn accumulated the most dry matter, but accumulated dry matter could be directly compared to the control or short deferred period beyond February because of the longer than expected grazing rest that occurred in these two treatments after

February. For both trials, the control treatment initially had a lower leaf dry matter, but the dry matter of all treatments decreased to similar levels once grazing or cutting of treatments resumed. However, by the end of the glasshouse trial, the control treatment appeared to be performing better than treatments with longer length periods of deferment, whilst the opposite was the case for the field-based trial.

Some uncertainty is added to these results due to the control treatment for both trials being subjected to a longer than intended interval between grazing or cutting from February to May, alongside deferment treatments that had resumed either standard grazing or cutting. This rest period helps explain the autumn accumulation of dry matter seen in the field trial, but no increase in biomass was seen in the glasshouse for the corresponding period. However, in the field, the percentage ground cover of broadleaf weed was much higher by May.

The main weeds observed were plantain. Plantain is categorised as a weed for the purposes of this study as it was not intentionally sown, however plantain can give higher dry matter yields over autumn when compared to perennial ryegrass based pastures (Moorhead & Piggot, 2009). The ground cover percentage for ryegrass did not change significantly from before or after the longer rest period, but trended lower in July in treatments with longer periods of deferment. While differences were not statistically significant in July for ground cover, the coinciding biomass measurement in the glasshouse also had the same trend with lower leaf biomass for treatments with longer periods of deferment. Based on these results it is possible that the percentage dry matter for ryegrass decreased for both trials in July. Results from previous research found improved dried matter yields after periods of deferment were applied to a pasture (McCallum et al., 1991), while others that focused on perennial ryegrass had variable results and were less conclusive (Graham et al., 2000; Korte et al., 1984).

The results of the field based trial here support previous work that a period of deferment over summer or autumn can give increased dry matter yields in pasture. However, the best timing for deferment period is uncertain due to the long grazing rest for the control. How long this effect of higher dry matters lasts wasn't determined in the field based trial, but similar studies showed promising long term

results for up to a year, even under dry summer conditions (Tozer et al., 2020). Consideration of these results suggest a positive effect in dry matter yield may be achieved by a period of deferment but pasture composition will be dependent on seasonal conditions and what is in the seedbank (Tozer et al., 2010). While the seedbank and interspecific competition could influence the post-deferment outcome by changing pasture composition, this was not the case for the monocultures in the glasshouse. Based on the results from both trials, dry matter yield of perennial ryegrass was not increased following a period of deferment even when competition between other species was excluded.

There were no significant changes in the number of live tillers counted for the glasshouse trial even when above ground biomass differed between treatments. However, the percentage of dead tillers was higher for longer deferred treatments. Consideration of these results suggest two possible outcomes. One, a greater rate of tiller appearance must have occurred in the longer deferred treatments to replace the higher numbers of dead tillers. Two, the weight of tillers may differ if the biomass of deferred treatments was lower but live tiller number was not. While tiller densities were not measured in the glasshouse trial, they were recorded in the field trial. For the corresponding time period in May, tiller densities were higher in the control and Def x14, but by July the control had lower tiller density than treatments that had had a period of deferment. Other literature has found tiller density to decrease over a reproductive period but after a grazing cycle, there were increased tiller densities and increased tiller appearance for treatments where grazing had been withheld over spring, tiller weights were also found to decrease in autumn with increasing tiller density (Hernández-Garay et al., 1993; Hernández-Garay et al., 1997; Korte et al., 1984). While this means tiller weight can change over time it is not certain if this is the effect that happened in the longer deferred treatments for the glasshouse trial or the reason why biomass decreased but total live tiller number didn't. However, despite the complication of the long grazing rest before May, tiller densities were positively influenced for both the previously deferred treatments over autumn compared with the control. This suggests a tillering effect in relation to a period of deferment did occur, though the extent of the interaction between tillering and deferment length is unknown.

Seedling establishment following the reproductive period is another area of consideration when managing a period of deferred grazing. Previous work found seedling establishment could increase tiller densities and dry matter through late summer and early winter, however mortality of seedlings is increased by competition with existing tillers, and grazing too soon will prevent nearly all natural reseeding of pasture (L'Huillier & Aislabie, 1988). Timing of grazing to support seedling recruitment is one way that deferred grazing could support pasture persistence. There were no visual observations of seedlings made at the field site in this trial. For the results of this study, it is possible that the opportunity for deferred grazing to support seedling establishment was missed because of the drought that was followed by a longer grazing interval.

#### **5.4 Production**

The final implications of this study lie in whether using a period of deferred grazing will benefit farmers in improving farm productivity. Feed quality is a measure of both the overall intake of feed and the nutritive value (Waghorn & Clark, 2004), so for farm productivity to be improved, either or both the amount of feed supplied and its nutritive value needs to be increased. Higher dry matter yields alone for grazings following a period of deferment are not necessarily enough to improve farm productivity.

Nutritive value falls even as dry matter increases when pasture species become reproductive (Čop et al., 2009). The control treatment had higher crude protein % than deferred treatments. CP content over 20% exceeds the dietary requirements of cows (Fulkerson et al., 2007). As CP in this trial did not reach values this high, the control treatment did offer a higher value feed than deferred plots for the same weight of dry matter. Considering the higher dry matter available it appears the deferred treatments still offered a valuable feed supply after the deferment period. With respect to the missed grazing opportunities to achieve higher standing dry matter, this feed source may not prove to be more productive for livestock but could offer greater resilience to the farm system by offering a feed supply at a time when climate is unfavourable to growth. This was particularly the case when the fourteen week deferred treatment was opened up at the end of the drought period, allowing this paddock to provide a feed supply at a critical time when dry

matter elsewhere on the farm was low. In situations where feed supply is limited over winter, the autumn accumulated dry matter in the long deferred pasture could also be beneficial. While deferred plots had low nutritive values for the initial grazing after opening, nutritive value of the deferred plots returned to similar values to the control after one grazing cycle.

The deferred plots both missed two grazing events in November and December. It is less clear how the value of the increased feed supply from deferment compares to the value of the additional grazing periods of the control plots. What can be suggested from the results is that any loss in nutritive value by deferring paddocks does not have a long-term negative impact on feed supply. Previous literature has highlighted the drop in nutritive value and therefore recommend that pastures are kept vegetative for improved farm production (Michell et al., 1987). The results here give some confidence that if feed is at a surplus on farms over the spring period, paddocks can be deferred with minimal concern for lost productivity. This is further supported by previous studies on deferred grazing that have resulted in positive responses from farmers for providing a feed supply at the end of summer and in early autumn when feed elsewhere may be more scarce (Tozer et al., 2020).

The main way that deferring paddocks from grazing can confidently contribute to farm production is if the persistence of desirable species is improved over a long enough period to improve feed quality. In this study, the species of focus was perennial ryegrass which has limited drought tolerance (Jiang & Huang, 2001). Considering results from the beginning and end of the trial, little benefit of deferred grazing was seen for perennial ryegrass. Percentage ground cover for ryegrass was lower and broadleaf weeds higher by July than in the preceding November, while in the glasshouse, above ground biomass was not improved in the long term by a deferment period. Some improvement was observed in tiller densities in the field which were higher in deferred treatments than the control in July but this result could not be investigated further due to the trial ending. Overall, this study provides insufficient evidence to suggest pasture persistence of ryegrass is supported by deferred grazing.

In contrast, plantain was higher in all plots from autumn onwards. Plantain is categorised as a weed for the purposes of this study as it was not intentionally



sown, however it does hold value to livestock as a feed source (Stewart, 1996). This means the paddock productivity was still high after the deferment period and would have contributed to the higher dry matter and nutritive value rates seen for autumn measurements. This combined with the later benefits in production found in the paddock suggests deferred grazing has potential for improving farm productivity, but more research is needed to determine the effect on longer term pasture persistence, especially under extreme climatic variables such as prolonged drought.

## **5.5 Further Research**

Several further areas of research and questions have been opened up by this study. Some next steps to consider would be to obtain root cores from a deferred pasture with a non-limiting soil type to investigate rooting depth and if the temporary increase in biomass in the lower levels found in the glasshouse also occurs in the field. Using other methods of root measurements such as rhizotrons could also be beneficial to monitor root dynamics and bring more certainty to the results.

To answer questions about if heat or nutrient stress affected ryegrass growth a repeat experiment under controlled conditions with these variables accounted for would be beneficial for more thoroughly testing whether rye grass root systems are benefited by deferment.

Further research into the influence on other species, and pasture composition changes with drought would help with the understanding of what limits for prevalence of other species or climate conditions are needing to benefit ryegrass over other pasture species.

In consideration of the drought effects, further investigation could be done to discover if dry matter yield or pasture persistence is improved when more drought tolerant species are sown. Research into the timing of deferring a pasture in relation to drought could also be useful to find if there are benefits to pasture having a larger or deeper root mass before a drought period.

Following the longer grazing rest both the control and previously deferred plots appeared to have higher tiller densities. It could be used to perform a controlled study on tiller interactions following a short deferment over the reproductive

period, followed by a grazing than another deferment period to help quantify the variable effects of changes in length to a grazing cycle. This might be especially significant if timed to consider seedling establishment.

Further research that would be of use to farmers would be to consider the farm system effects of deferred pasture with combined variables of yield, timing of deferment and opening as a feed supply as well as nutritive value.

## **5.6 Conclusion**

Overall, the results here suggest that withholding grazing over the reproductive period of late spring through summer or to autumn may have some positive benefits in dry matter yield for the paddock. Above ground biomass was also higher in February for the glasshouse based experiment but resulted in no lasting increase in ryegrass yield after deferment.

Deferring to autumn resulted in higher accumulated dry matter but lower nutritive value, both deferred treatments had similar densities however the short deferred could not be fairly compared to the long deferred at this time point due to the effects of the long grazing rest. As a result, a definitive answer on the comparison of a longer or shorter deferment cycle cannot be inferred from the results. Benefits found for perennial ryegrass were limited once grazing or cutting of treatments resumed. Tiller densities in the field appeared higher for deferred treatments in July compared to the control but there were no lasting benefits in tiller number found in the glasshouse trial. Deferment length did not have a significant effect on root biomass in either trial.

In consideration of uncertainty in the trial brought on by drought stress and the long grazing cycle that occurred from February to end of April as well as what has been found in previous literature, it is possible there may be further benefits to deferring a pasture that are not accounted for by these results. However, this is dependent on other variables such as climatic conditions, soil type and nutrient availability and pasture species presence. Perennial ryegrass cannot be expected to benefit from a period of deferment if other variables restrict growth or ability to compete against other species. Further research is needed to determine the effect

of different lengths of deferment on roots, tillers or species interactions in hill country pasture.

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